# STUDIES ON ENTOMOPATHOGENIC NEMATODES AND THEIR BIOCONTROL POTENTIAL AGAINST *HELICOVERPA ARMIGERA*

**THESIS**

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IN

ZOOLOGY



BY

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## Certificate

This is to certify that the research work presented in this thesis entitled "**Studies on entomopathogenic nematodes and their biocontrol potential against *Helicoverpa armigera***" by **Mohammad Abid Hussain** is original and was carried out under our supervision. We have allowed Mr. Hussain to submit it to the Aligarh Muslim University, Aligarh in fulfillment of the requirement for the degree of Doctor of Philosophy in Zoology.


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# Contents

# 1 Introduction

The gram pod borer, *Helicoverpa* (=*Heliothis*) *armigera* (Hübner) (Lepidoptera: Noctuidae) is a widespread polyphagous pest species of many agricultural and horticultural crops (Reed & Pawar 1982; Zalucki et al. 1986; Fitt 1989; Gowda 2005). This pest has been recorded feeding on 182 plant species across 47 families in the Indian subcontinent, of which 56 are heavily damaged and 126 are rarely affected (Pawar et al. 1986). Economic losses of up to Rs.1,000 crore due to this pest alone have been reported in crops like cotton, pigeonpea, chickpea, groundnut, sorghum, pearl millet, tomato, and other crops of economic importance (Raheja 1996). Barwale et al. (2004) estimated about Rs. 1,200 crore worth of pesticides are used in India to control the bollworm complex of cotton. Whereas *Helicoverpa* causes an estimated loss of US$ 927 million (equivalent of Rs. 4,580 crore) in chickpea and pigeaonpea, and possibly over US$ 5 billion (≈ Rs. 24,705 crore) on different crops worldwide (Sharma 2001).

The status of *Helicoverpa* as a serious pest of agricultural crops is due to its high mobility, polyphagy, facultative diapause as pupae, rapid generation turnover, fecundity and predilection for harvestable parts of high-value crops such as cotton, tomato, pulses and cereals (Fitt 1989). Management strategies for *Helicoverpa* include cultural management of the crop and its environment, biological control using predators, parasitoids and microbial pesticides, sex pheromones for population monitoring or mating disruptions, host plant resistance and chemical control (Gowda 2005). However, pest has become serious with regular outbreaks and has developed resistance to almost all conventional insecticides including synthetic pyrethroids (Armes et al. 1996; Kranthi et al. 2002). As a result, chemical control through the use of synthetic insecticides could not become panacea in the protection of agriculturally important crops. There is an increasing emphasis on integrated pest management (IPM) streategies with more reliance on biological control. IPM applies multiple methods to suppress pest populations, thereby reducing dependence on conventional insecticides, which can have unintended harmful consequences for the environment and human health (Dent 2000). Now, the use of microbial pesticides have therefore been considered a more sustainable and environmentally benign option to control *Helicoverpa* (King & Coleman 1989). Unfortunately previous control attempts using *Ha*NPV, *Bt*, NSKE, neem and its derived product were not found effective to a great extent and substantial reduction in degree of losses caused by this dreaded pest could not be achieved (Sachan & Lal 1997). The deactivation by UV light, insufficient titres ingested by larvae and lack of virulence could be the practical problems.

Entomopathogenic nematodes (EPN) of the genus *Steinernema* and *Heterorhabditis* (Rhabditida: Steinernematidae, Heterorhabditidae) are symbiotically associated with bacteria of the genus *Xenorhabdus* and *Photorhabdus* (Enterobacteriacae), respectively. They are lethal obligatory parasites of insects (Gaugler & Kaya 1990; Kaya & Gaugler 1993), yet pose no threat to plants, vertebrates and many invertebrates (Ehlers & Hokkanen 1996). Entomopathogenic nematodes possess many attributes of an excellent biological control agent. They have a broad host range and are virulent, killing the host rapidly (within 24-72 h). They can be produced in large quantities on artificial media and are easily applied with standard spraying equipments or irrigation systems (Shapiro-Ilan & Gaugler 2002).

Entomopathogenic nematodes received much attention during last two decades because of their potential use as biopesticides against insect pests. These nematodes can also provide effective control of some agriculturally important lepidopteran, coleopteran and dipteran pests (Ghode et al. 1998; Karunakar et al. 1999b; Banu & Rajendran 2002; Shapiro-Ilan & Gaugler 2002; Vyas et al. 2002). However, there is scant research work on pod borer control by the use of entomopathogenic nematodes. Therefore, the overall objective of the present study was to search an alternative biocontrol method of *H. armigera*. The strategy used during this study was to search indigenous entomopathogenic nematodes, to learn more about their activity and mode of action when they are applied alone for biocontrol of *H. armigera* and finally, to evaluate the bioefficacy of these nematodes in field application against *H. armigera*.

To achieve the goal, following specific studies were carried out:

- Isolation of entomopathogenic nematodes (EPN) from soil.
- Studies on infectivity of EPN against *Helicoverpa armigera.*
- Effect of temperature on survival of EPN and their infectivity to prepupa of *H. armigera.*
- Survival of EPN on pigeonpea/chickpea foliage after its application.
- Biocontrol of soil-dwelling life stage of *H. armigera* with EPN.
- In vivo production of EPN in host insects, i.e., *Galleria mellonella* and *Corcyra cephalonica.*
- Field application of EPN for the management of *H. armigera.*

It is expected that the knowledge obtained from this study will be used to facilitate the biocontrol of *H. armigera* – a dreaded pest of agriculturally important crops.

# 2 Review of literature: *Helicoverpa* and entomopathogenic nematodes (EPN)

## 2.1 Economic importance of *Helicoverpa*

*Helicoverpa armigera* (Hübner 1808) (Lepidoptera: Noctuidae), known by many common names such as cotton bollworm, corn earworm, tobacco budworm, old world bollworm, legume/gram pod borer, etc., is a very serious pest of economically important crops such as cotton, maize, chickpea, pigeonpea, and a range of oilseeds, vegetables and fruit crops (Gowda 2005). *H. armigera* is widely distributed in Asia, Africa, Australia and the Mediterranean Europe (Sharma 2001). A conservative estimate is that over US$ 1 billion (≈ Rs. 4,941 crore) is spent on insecticides to control this pest (Gowda 2005). Therefore, in addition to the huge economic losses caused directly by this pest, there are several indirect costs accruing from the deleterious effects of pesticides on the environment, as also human and animal health.

In recent times, crop production has been severely threatened by the increasing difficulties in controlling *H. armigera* (Kranthi et al. 2002). Several factors, including increasing levels of resistance to pesticides and rise in cropping intensity has contributed to greater importance of this pest (Shanower et al. 1998). Agronomic factors, such as high yielding varieties, increased use of irrigation and fertilizers, and large-scale production and planting of alternate crop hosts contribute towards greater prevalence and increased severity (Reed & Pawar 1982; Fitt 1989). However, regional and local differences in host preference can give rise to differences in pest status on particular crops, e.g., in northern and southern India, its severe infestations on cotton have been recorded only in the recent past. In northern India, *H. armigera* was unimportant as a pest of cotton till recently. Although infestation of chickpea in the same area is severe, and causes up to 90% pod damage (Sehgal & Ujagir 1990). Presently, it is a major pest of cotton in most of the cotton growing regions in India, Pakistan, and China (Sharma 2001).

*2.1.1 Host range*

*Helicoverpa armigera* is a highly polyphagous species. It has a wide host range, covering more than 180 plant species belonging to 45 families in India (Manjunath et al. 1989). The most important crop hosts of which *H. armigera* is a major pest are cotton, tomato, pigeonpea, chickpea, sorghum and cowpea. Other hosts include dianthus, rosa, pelargonium, chrysanthemum, groundnut, okra, peas, field beans, soybeans, lucerne, *Phaseolus* spp., other Leguminosae, tobacco, potatoes, maize, flax, a number of fruits (Prunus, Citrus), forest trees and a range of vegetable crops (Sharma 2001; CABI 2006).

*2.1.2 Extent of damage*

*Helicoverpa armigera* is the single most important insect pest of various crops such as cotton, pigeonpea and chickpea production in India. In cotton, damage caused by *H. armigera* has been estimated to be 10 to 30% in Gurajat and 45% in the northern zone (Sundaramurthy 1992). Coastal Andhra Pradesh suffered heavily from *H. armigera* damage in 1987, where synthetic pyrethroids were indiscriminately used on cotton (Reddy 1990). The outbreak of *H. armigera* in Andhra Pradesh resulted in heavy economic losses and also caused loss of human lives due to failure of crop. Yield losses from *H. armigera* in cotton ecosystems in the polycrop systems of Tamil Nadu and Karnataka have been estimated to range from 35 to 38% (Satpute et al. 1988). In 1995-96, outbreaks of *Helicoverpa* in eastern Uttar Pradesh have caused economic damage to the farming community where losses in chickpea seed yield varied from 75 to 90% (Lal 1996). The farmers of Azamgarh district were worst hit as in many places there was 100% loss in seed yield.

In pigeonpea, yield loss ranged from 470 to 988 kg/ha, which accounts for 29 to 85% of grain yield in different cultivars. A single *H. armigera* larva per 10 plants reduced pigeonpea yields by 31 kg/ha (Venugopal Rao et al. 1992). More than 20% damage was recorded in Punjab, Madhya Pradesh, Bihar, Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu. Moderate damage (7 to 20%) has been recorded in Haryana, Rajasthan, Uttar Pradesh, Gujarat, Orissa and West Bengal, and low damage (< 7%) in the north-eastern states, where pigeonpea is less widely grown (Sachan 1992).

On cotton, 2 to 3 larvae per plant can destroy all the bolls within 15 days; on maize, they consume grains; on tomatoes, they invade flowers and fruits,

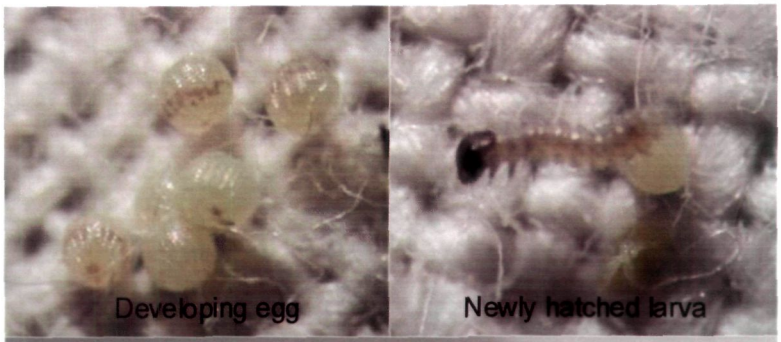
and lead to fruit drop. In maize, a single larva damages 2 to 34 grains, causing a loss of 1.9 g in grain weight and yield loss of 1.5%. In pigeonpea, one larva per plant reduces 5 green pods, 7 dry pods, 18 grains, 3.8 g pod weight, and 2.1 g grain weight per plant. A unit increase in larvae per plant results in 2.6 and 4.9% increase in pod damage at the green and dry stages, respectively (Meenakshisundaram & Gujar 1998).

*2.1.3 Morphology, biology and ecology*

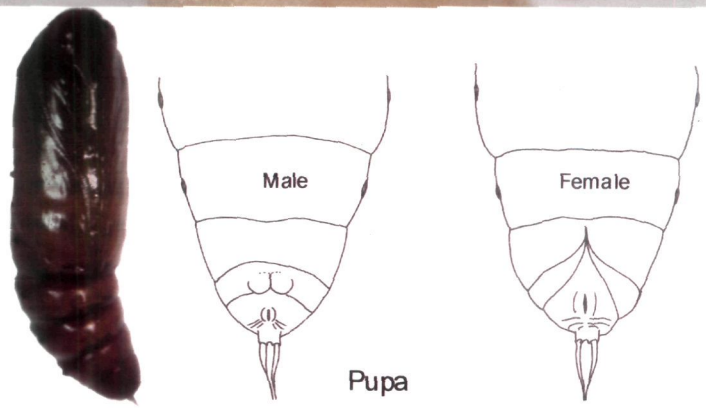
The major pest status of *Helicoverpa* is because of its high mobility, polyphagous nature, high reproductive rate and ability to undergo diapause. *H. armigera* larvae preferentially feed on reproductive structures, which provide enhanced growth and fecundity relative to feeding on vegetative parts. In addition, *H. armigera* feeds on a diverse array of host plants, is a strong disperser, and undergoes facultative diapause (Fitt 1989). All of these life history features contribute to make *H. armigera* one of the 'world's worst pest' (Pimbert et al. 1989).

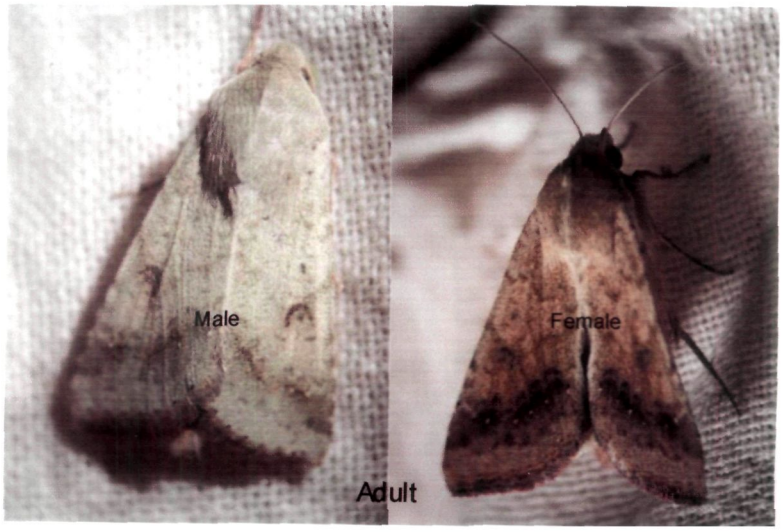
*Helicoverpa* is easily diagnosed by the long coiled vesica with a strip of cornuti along its length, and a long alternately dilated and constricted appendix bursae in the female whereas males of *Helicoverpa* species have a patch of modified scales on the prothoracic femur (Matthews 1999).

The biology of *H. armigera* is typical of the Noctuid insect. Morphology of various life stages of *H. armigera* have been described by Kirkpatrick (1961), Hardwick (1965), King (1994), and Matthews (1999) in greater details, which are narrated below. Life cycle consists of four stages, namely, egg, larva, pupa and adult (Figure 2.1).


Developing egg
Newly hatched larva


Final instar larva


Male
Female
Pupa


Male
Female
Adult

*Eggs*: Eggs are sub-spherical with a flattened base or pomegranate-shaped (dia. 0.4 to 0.6 mm). Freshly laid eggs are whitish or creamy-white, which develop a central, reddish brown band as the embryo develops. This gradually darkens, together with the rest of the eggs, which becomes dark-brown before hatching. Infertile eggs are white or yellowish white, and become yellow and cone-shaped as they desiccate.

A female may lay up to 3,180 eggs mainly at night (457 in 24 h), singly on its host plants. Oviposition period lasts for 5 to 24 days. The duration of the egg incubation period depends on temperature, and varies between 2 to 5 days (usually 3 days) (Reed 1965; Singh & Singh 1975; Jayaraj 1982; Rajagopal & Channa Basavanna 1982).

*Larvae*: Newly emerged larvae are translucent, yellowish white, with faint gray longitudinal lines. Prolegs are present on the third to sixth, and tenth abdominal segments. The second-instar larva is similar to the first-instar larva, with slight darkening of colour and some lightening of the sclerotized head capsule, thoracic and anal shields, and thoracic legs. The third-instar larva is characterized by two colours, having either a green or a red-brown ground colour, with a greenish-fawn or cream to fawn coloured head capsule. The characteristic patterns of the larva become more prominent and darker in later instars, although this depends to some extent on the host plant.

The full-grown larva is about 35 to 42 mm long, and the integument has a characteristic granular appearance, consisting of close-set minute tubercles. The head capsule is mottled, light-brown to reddish-brown, and the prothoracic and anal plates are pale brown. The ground colouration of the last instar is highly variable, ranging from blue-green to yellow, pink and reddish

brown, with the density of the longitudinal lines. The background colour of the host, interaction of light, temperature, food, and heredity determine the colour variation. Larvae on sunflower heads are often pale yellow, whereas those on chickpea are frequently uniformly green, with a few exceptions. Preferential predation of contrasting colour types may be one of the factors resulting in preponderance of one type of larvae on a host plant. However, such correlations are by no means fixed as larvae of all colour forms may be found on all types of host. The colour of the dorsal anterior cervical shield hairs may be used to distinguish between sixth instar, e.g., *H. armigera* – white, and *H. punctigera* – black (Zalucki et al. 1986). However, the only reliable method for separating younger stages is by electrophoretic techniques (Daly & Gregg 1985). The number of larval instars varies from five to seven, with six the most common. Hardwick (1965) found that 30% of the larvae matured in 5 instars, 69% in 6 instars, and 1% in 7 instars.

The young larvae usually eat some or the entire empty eggshell before feeding on the host plant. Newly emerged larvae move around for some distance, with occasional feeding on the surface before settling down at a preferred site (flower bud or flower in cotton and pigeonpea, young leaves in chickpea, corn silks, and the soft grain or young whorl leaves of cereals). Older larvae prefer flower buds and young cotton bolls, legume pods, or cereal grain, although leaves are also eaten when plants are small or only a few fruiting bodies are present. The large larvae normally feed with the front portion of its body inside the fruiting bodies, with the remainder protruding outside (Figure 2.2). At times, the larvae may be entirely hidden inside the square, boll or the pod, with only a small hole lightly spun over with silk

threads to indicate its presence. Most flower buds, bolls or pods, which have been attacked, show an accumulation of faeces. This may render the larva prone to discovery and attack by the natural enemies responding to the semio-chemicals from the host plant. Molting often takes place on the upper surface of the leaf in full sunlight, possibly to hasten drying of the new cuticle (Reed 1965). Larvae tend to move between feeding sites and adjacent plants, especially when preferred food is limiting, and population densities are high. Encounters between older (> 3$^{rd}$ instar) larvae usually result in cannibalism, often resulting in only one large larva per flowering bud, boll, pod, whorl leaves, or a panicle.

The duration of larval development depends not only on the temperature, but also on the nature and quality of the host plant. Larval development varies between 15 days on maize to 24 days on tomato at 23.3°C (Rajagopal & Channa Basavanna 1982). The average larval period of 21 days was recorded on cotton flower buds (including a pre-pupal period of 3 days) at 21 to 27°C (Reed 1965). Under laboratory conditions, larval period ranges from 17 to 20 days, with a minimum and maximum on pigeonpea and tomato, respectively (Dhandapani & Balasubramanian 1980). However, Singh & Singh (1975) reported that larval period ranges from 8 to 12 days on tomato, 18 days on cotton and maize. The weights of fully-grown larvae differ considerably with diet. The heaviest larvae have been observed on cotton, and the lightest ones on tomato and sorghum (Jayaraj 1982).

Figure 2. Characteristic *Helicoverpa armigera* damage in chickpea and pigeonpea pods

*Pupae*: On completion of larval development, the larvae drop or crawl to the ground and enter the soil for pupation. The depth at which the pupal cell is formed varies considerably, depending on hardness and wetness of the soil, presence of cracks and crevices, and surface litter. Pupation takes place at a depth of 2.5 to 17.5 cm (Jayaraj 1982). Occasionally, pupation may also take place inside a tunnel in a maize cob (Reed 1965) or on the soil or leaf surface. The larvae spin a loose web of silk before pupation. Pre-pupal stage is shorter and stouter, with a uniform color, and lasts for 1 to 4 days.

The pupae are 14-22 mm long, and 4.5 to 6.5 mm in width across thorax. Pupae are mahogany-brown, smooth-surfaced, and rounded both at the anterior and posterior ends, with two parallel spines at the posterior tip. The distance between the bases of these spines can be used as a criterion to separate *H. armigera* from *H. punctigera* (>0.22 mm in *H. armigera,* and <0.20 mm in *H. punctigera*) (Zalucki et al. 1986). There may be considerable variation in pupal size as a result of larval food quality. Female pupae generally are heavier than the male pupae.

*Helicoverpa armigera* exhibits a facultative diapause, which enables it to survive the adverse weather conditions in winter as well as in summer. The duration of the pupal stage therefore depends on whether or not diapause has been induced during the earlier life stages. In the non-diapausing pupae, the pupal period ranges from about 6 days at 35°C to over 30 days at 15°C. Pupal period may also vary with the host plant on which the larval development has been completed (Jayaraj 1982). In the diapausing pupae, the pupal period may last for several months. Temperatures of over 30°C during pupation produce pale coloured adults.

*Adults*: The adult are stout-bodied moths, with a wingspan of 35 to 40 mm, and body length of 18 to 19 mm. The general colour varies from dull greenish-yellow, buff to olive-grey, and light brown to blackish markings on the wings. Males *H. armigera* are uniformly pale cream, and forewings generally tinged with green. Females are darker, and the forewings are without a green tinge.

Longevity of adults depends upon the availability of food such as sucrose or nectar, pupal weight (fat body content), temperature, adult behaviour such as flight activity. Female moths generally live longer than the males. Adult lifespan is determined by the availability of food. In the absence of suitable food source, depletion of the fat bodies is rapid, and death occurs in a few days (Armes 1989). Mean and maximum longevity at room temperature (21 to 27°C in winter and 27 to 32°C in summer) is similar. Predation, disease, and abiotic factors may also reduce adult longevity under natural conditions.

The developmental threshold temperatures for egg, larval and pupal stages are 10.8, 13.6, and 14.6°C, respectively (Qureshi et al. 1999), and effective temperature sums for development being 45.5, 200 and 143 day-degrees, respectively. Its life tables have been studied by Patel & Koshiya (1997ab) on pearl millet and lucerne. On pearl millet, the population increased by 0.1423 at a finite (lambda) rate of 1.156 females per female per day (Patel & Koshiya 1997a). On Lucerne, the maximum duration of egg, larval, and pupal stages was 3, 17 and 15 days, respectively (Patel & Koshiya 1997b). The net reproductive rate represented by total number of female births was 206.3. The population increased (rm) by 0.1272 at a finite (lambda) rate of 1.136 females per female per day. Mean generation time being 42 days. At a

stable age distribution, *H. armigera* population would comprise of 4.3% eggs, 52.7% larvae, 5.4% pupae, and 0.6% adults.

*Movement and dispersal*: The importance of *H. armigera* is largely due to its well-developed survival strategies, diapause, and dispersal, which enable it to exploit food sources separated by unfavourable times and distance (Pedgley 1985; Farrow & Daly 1987; Pedgley et al. 1987; Fitt 1989; Riley et al. 1992; King 1994). *H. armigera* is a facultative migrant, and responds largely to local environmental cues and undertakes short or long distance flight in the direction largely governed by prevailing weather systems (Fitt 1989). In diverse habitats such as India, the tendency to fly may have been moderated by feeding, which reduce the pre-maturation period. Adults can migrate over long distances, e.g., from southern Europe to the UK (Pedgley 1985).

*2.1.4 Control measures*

Pest management strategies for *Helicoverpa* require integration of different control tactics to implement a threshold based on the relationship between population density and economic loss. Tactics which can be deployed for minimizing losses include cultural manipulation of crop and its environment; host plant resistance, including wide hybridization and transgenics; biological control, including use of microbial pesticides; sex pheromones for population monitoring or mating disruption; and chemical control (Bottrell 1979; Gowda 2005).

***(a) ETL and population monitoring through pheromone***

Field monitoring of pest populations is necessary to determine whether the threshold has been exceeded, and control measures should be undertaken. Location-based economic threshold level (ETL) for *H. armigera* in cotton were

reported by Simwat (1994) as 25 adults/trap/night or 1 larva/10 plants or 20 balls at Bapatla and Coimbatore; 10% incidence in reproductive parts at Bhatinda; 2-7 larval unit/10 plants at Surat.

*Helicoverpa armigera* populations can be monitored through pheromone-baited traps to understand the onset infestations, and study the population dynamics (Nesbitt et al. 1979). Catches in pheromone-baited traps exhibit a negative but non-significant correlation with temperature, wind speed, sunshine and rainfall; and a significant positive correlation with maximum relative humidity (Dhawan & Simwat 1996). Monitoring the population of bollworms helps in the early detection of infestations, and their build-up depending on abiotic factors.

***(b) Cultural control***

*Early/timely planting*: Early and/or timely planting of crops can help avoid periods of peak abundance of *H. armigera*. The planting times are often decided by the rainfall pattern and availability of soil moisture. Late planted crops often suffer greater pest damage because of build up of pest populations over the cropping season. Early plantings of pigeonpea in India (Dahiya et al. 1999) result in reduced damage by *H. armigera*.

*Cropping season*: The use of short-season cultivars has often been used to avoid pest damage, and has been used effectively to minimize bollworm damage in cotton in USA. Plant growth regulators have also been used to shorten the crop maturity (Bradley et al. 1986). Early termination of flowering and fruiting can also check the population carryover from one season to another or reduce the number of generations.

*Intercropping*: A careful selection of a cropping system can be used to minimize losses due to insects for sustainable crop production. Intercropping chickpea with mustard or safflower (Das 1998), pigeonpea with cowpea (Hegde & Lingappa 1996) and sorghum (Mohammed & Rao 1998), and tomato with radish (Patil et al. 1997) results in reduced damage by *H. armigera*. Intercropping can also be used as a means of encouraging the activity of natural enemies. Crops that can serve as perches (e.g., sunflower in chickpea) for insectivorous birds such as drongo can also be used to increase the predation by them.

There is some evidence that intercropping pigeonpea with short-season legumes such as soybean, mungbean or sorghum reduces the influence of *H. armigera* on pigeonpea. Under heavy infestations, manually shaking pigeonpea plants to dislodge larvae is often resorted to by farmers in south India (Rao et al. 2005). Identifying promising intercrop systems in pigeonpea, chickpea, groundnut and cotton in regulating *Helicoverpa* populations have shown mixed results. Certain intercrops such as groundnut and coriander in chickpea were found to be beneficial in reducing the incidence of insect pests, besides the additional income obtained through intercrops (Sekhar et al. 1995). Chickpea plots sown with coriander had 6 *Campoletis chlorideae* Uchida (Ichneumonidae: Hymenoptera) pupae in 5-m row compared to 2 parasitized larvae in the sole crop (Turkar et al. 2000). Chickpea intercropped with wheat, mustard or safflower suffered less damage by *H. armigera*, and had higher yields than chickpea intercropped with linseed, lentil, or pea. Chickpea alone suffered maximum damage. Strip and adjacent cropping of pigeonpea with sorghum reduced the population of insects and, consequently

damage to pigeonpea, primarily due to the influence of natural enemies and their movement from sorghum to pigeonpea (Pawar 1993).

*Trap crops*: Trap crops and diversionary hosts have been widely used in the past to reduce the damage by *H. armigera*, but have seldom been successful (Fitt 1989). Although infestation of cotton by *H. armigera* has been reduced by late-planted maize and sorghum (Nyambo 1988), their comparatively short attractive periods, and the potential of earlier planted crops to increase pest populations are the major disadvantages. Sunflower, marigold, sesame, and carrot have been used as trap crop for *H. armigera* control.

*Destruction of pupae*: Removing or ploughing the cotton stubble can be used to reduce over-wintering populations of *H. armigera* (Fitt & Forrester 1988). Soil moisture and flooding with water also affect pupal survival and moth emergence (Murray & Zalucki 1990ab). Irrigation or flooding of the fields at the time of population (Yang et al. 1999) reduces the pupal survival, and thus leads to decreased population densities in the following generation or season.

***(c) Host-plant resistance***

The development of crop cultivars resistant or tolerant to *H. armigera* has a major potential for integrated pest management (Lal et al. 1986; Fitt 1989; Sharma et al. 1999), particularly under subsistence farming in the developing countries. Development of crop varieties that are resistant or tolerant to *H. armigera* has received the major attention in the recent past, particularly for cotton, pigeonpea and chickpea. Many crop species possess some genetic potential, which can be exploited by breeders to produce varieties that are less susceptible to *H. armigera* damage.

*(d) Chemical control*

There is substantial literature on comparative efficacy of different insecticides against *Helicoverpa* on cotton, pulses, vegetales and other crops. Information on chemical control has been reviewed extensively by Zalucki et al. (1986) and Matthews (1989). The early history of chemical control of corn earworms is given by Hardwick (1965), while COPR (1983) includes a list of 29 insecticides effective for the control of *Helicoverpa* spp. at the recommended rates.

Most insecticide applications are targeted at the larval stages. However, larvicides have only a limited role to play in *Helicoverpa* management. Control measures directed at adults and eggs (and neonate larvae) are most effective in minimizing the *Helicoverpa* damage. Young larvae are difficult to find, and older larvae soon burrow into the floral organs where they become less accessible to contact insecticides, require higher doses to kill, and cause direct economic loss. In insecticide resistant populations, the larvae were still susceptible when these were less than 4 days old, so targeting neonates is essential in areas where resistant populations are encountered (Daly et al. 1988). The most effective control has been achieved by spinning disc ULV applicator as compared to mist blower (Parnell et al. 1999), and spray initiation at 50% flowering has been found to be most effective (Singh & Gupta 1997).

*Insecticide resistance management*: Extensive studies carried out by Armes et al. (1996) on *H. armigera* collected between 1991-1995 from 51 locations of Indian subcontinents indicate that resistance to some pyrethroids is ubiquitous in this pest. The resistance levels to cypermethrin ranged from 5 to 6,000 fold

and to fenvalerate 16 to 3,200 fold. Pyrethroid resistance levels were highest in central and southern India which are major cotton and pulse growing areas where excessive application of insecticides is common. Insecticide resistance management strategies have been aimed either to prevent the development of resistance or to contain it. All rely on a strict temporal restriction in use of pyrethroids and their alteration with other insecticide groups to minimize selection for resistance (Sawicki & Denholm 1987). Because of economic advantages, low toxicity to mammals and to some parasites and predators (King & Coleman 1989), much effort has been directed towards developing management strategies aimed at prolonging the use of synthetic pyrethroids, including the formation of manufacturers associations in different countries.

Studies on several field-collected cotton bollworms resistant to pyrethroids in China have shown that resistance is unstable. It declines rapidly without contact with the pyrethroids at first and then stabilizes at a 2 to 9-fold level (Wu et al. 1996), but it is difficult to recover susceptibility completely. The reduction in resistance during over-wintering and the 1st and 2nd generations is affected by the lower fitness of resistant *H. armigera* to low temperature and disadvantages in reproduction (Han et al. 1999).

***(e) Biological control***

The prospects for long-term biological control of *Helicoverpa* are discussed by King & Coleman (1989), and clearly this should be an important component of any regional Integrated pest management (IPM) programme. IPM utilizes two or more control measures together in an integrated manner to make maximum use of natural mortality factors, especially native predators and parasites. For any successful IPM, one must have complete

understanding of the factors that regulate pest population. Of utmost importance is the knowledge of biology and how physical factors such as temperature, humidity, rainfall, and natural enemies regulate the pest population. Every effort should be made to take advantage of weak points in the biology of the pest to devise the control measures.

Information on natural enemies and biological control of *H. armigera* has been reviewed by several workers in the recent past (King & Coleman 1989; King 1994; Romeis & Shanower 1996). In India, 75 arthropod parasitoids and 33 predators have been reported to occur on *H. armigera* (Manjunath et al. 1989). Sharma (2001) has published the most recent update on arthropod natural enemies of *H. armigera* in India including spiders, bird and other micro-organisms. In most areas, species of *Telenomus* and Trichogrammatidae (*Trichogramma* and *Trichogrammatoidea*) are important egg parasitoids, whereas at least one species each of Braconidae, Ichneumonidae and Tachinidae are larval parasitoids.

*Effect of host plant on parasitism*: Host-related differences in the activity of natural enemies have been recorded in case of *H. armigera* (Bhatnagar et al. 1982; Pawar et al. 1986; Zalucki et al. 1986; Manjunath et al. 1989). The influence of host plant is often more pronounced on egg parasitism, which is generally greater on cereals. The ichneumonid, *Campoletis chlorideae* is probably the most important larval parasite in India, but parasitism is affected by the host plant, and varies from 46% on sorghum to 3% on pigeonpea (Pawar et al. 1986).

The average rates of parasitism of the eggs of *H. armigera* (mainly by *Trichogramma* spp.) were 33% on sorghum, 15% on groundnut, and 0.3% on

pigeonpea (Pawar et al. 1986). In Gujurat, *T. chilonis* parasitized up to 98% of *H. armigera* eggs on tomato, potato and lucerne, but no egg parasitism was recorded from chickpea, probably because of the acid exudates secreted by the leaves (Manjunath et al. 1989). Bhatnagar et al. (1982) could record only 0.1% egg parasitism on pigeonpea. The parasites attacking *H. armigera* on sorghum in a sorghum/pigeonpea intercrop (mainly *Trichogramma* sp. and *Campoletis chorideae*) did not parsaitize *H. armigera* on the later maturing pigeonpea, where parasitism was chiefly by the tachinid, *Carcelia illota* (Bhatnager et al. 1982).

*Predation*: In general, predators have received less attention than parasites as natural control agents. The most common predators of *Helicoverpa* include *Chrysopa* spp., *Chrysoperla* spp., *Nabis* spp., *Geocoris* spp., *Orius* spp., *Polistes* spp. and various Pentatomidae, Reduviidae, Coccinellidae, Carabidae, Formicidae and Araneida (Greathead & Girling 1982, 1989; Zalucki et al. 1986; Romeis et al. 1999). Some predators have been used in augmentative release studies, notably *Chrysoperla carnea* (Ridgeway et al. 1977). Although effective in large numbers, high cost of large-scale production precludes its economic use in biocontrol of *H. armigera* (King et al. 1986).

*Entomopathogens*: There has been considerable interest in the innundative biological control using *Bacillus thuringiensis* and *Heicoverpa armigera* nucleopolyhedrovirus (*Ha*NPV) (Jayaraj et al. 1989) including entomopathogenic fungi and nematodes. *H. armigera* is highly susceptible to *Ha*NPV (Rabindra & Jayaraj 1988) and can be successfully controlled if the application coincides with the occurrence of early stages of the larvae, however, late stage larvae are more tolerant to the virus (Rabindra &

Subramaniam 1974), although to date, these tactics have not provided a viable alternative to insecticides.

Granular formulations of Bt, based on wheat meal and yeast extract (as phagostimulants), protect Bt against environmental degradation, and the formulation has been found to be more effective than Bt sprays (Navon et al. 1997). Under field conditions, *Beauveria bassiana* at 2.7 X $10^7$ spores/ml resulted in 6% damage in chickpea compared to 16.3% damage in the untreated control plots (Saxena & Ahmad 1997). Cai et al. (1997) reported avermectin (abamectin) as most effective to control *H. armigera* in the field.

Four sprays of *Ha*NPV were effective on groundnut for *H. armigera* control (Butani et al. 1997). The $LC_{50}$ value of *Ha*NPV has been found to be lower for the fenvalerate and endosulfan resistant strains than for the susceptible strain (Goud et al. 1997). Jaggery (0.5%), sucrose (0.5%), egg white (3%), and chickpea flower (1%) were effective in increasing the activity of *Ha*NPV (Sonalkar et al. 1997, 1998). Adjuvants - liquid soap (Ranipal 0.5%), indigo (0.2%), urea (1%), and cottonseed extract also increased *Ha*NPV activity.

Records of nematode parasites, usually Mermithidae, are available from all regions where inventories of natural enemies are available. However, high rates of parasitism occur only sporadically under favourable conditions. There is some evidence that, in India, they may be important in suppressing early-season populations on wild hosts (*Acanthospermum hispidum*), and groundnut on Alfisols (Bhatnagar et al. 1985). Parasitism of *H. armigera* larvae by *Hexamermis* sp. in south India was much higher on groundnut, tomato and some low growing weeds than on chickpea, sorghum and pigeonpea, where it was totally absent (Bhatnagar et al. 1985).

### *(f) IPM strategies*

In view of the need to make use of and exploit the existing spectra of natural enemies and to reduce excessive dependence on chemical control, particularly where there is resistance to insecticides, various IPM programmes have been suggested in which different control tactics are combined to suppress pest numbers below threshold level. These vary from judicious use of insecticides, based on economic thresholds and regular scouting to ascertain pest population levels, to sophisticated system, almost exclusively for cotton, using computerized crop and population models to assess the need, optimum timing, and selection of insecticides for sprays. The SIRATAC system, developed in Australia during 1980s, and its subsequent derivatives fall into this category (Hearn et al. 1981).

Singh et al. (2003) narrated a landmark report in cotton IPM and validation of the cropping system based on holistic community approach at village Ashta (1998–2001) in Nanded district (Maharashtra). The baseline information indicated less than a quintal average seed cotton yield per ha in *H. armigera* epidemic year of 1997 when the farmers had sprayed more than 12-13 rounds of chemical pesticide spray. All the farmers of the village were involved and the IPM approach covered 180 ha cotton area. The off-season practices included management of *H. armigera* on pigeonpea and chickpea through use of neem seed kernel extract (NSKE) and *Ha*NPV, field sanitation and deep ploughing. As the pre-sowing practices, multiplicity of cultivars was avoided by selecting only moderately resistant to sucking pest cultivars and treating the seed with imidacloprid for early crop growth stage pest. The sowing of the entire village was completed within a week to avoid vulnerability of crop over a

long period. The IPM interventions included use of *Trichogramma chilonis*, *Ha*NPV and NSKE. Lastly, chemical pesticides were used when needed and these included spray of endusulfan/bavistin for the management of bollworms or grey mildew in certain pockets. The average cost of crop protection inputs per year in the course of four years of the study was Rs. 1,298/ha in IPM compared to Rs. 1,890/ha in non-IPM. The average seed cotton yield in IPM was 1018 kg/ha with an average cost-benefit ratio of 1 : 1.88 compared to 649 kg/ha seed cotton yield and 1 : 1.14 C : B ratio in non-IPM. The system has become self-sustainable as the farmers of Ashta village have themselves become decision makers and on their own have started adopting many of the IPM practices.

A large number of trials were conducted to test the field efficacy of bioagents, entomopathogens, botanicals, chemicals and their combinations to control *H. armigera*. For examples, two sprays of Bt in combination with methomyl were most effective for the control of *H. armigera* on tomato, followed by methomyl and endosulfan. Mathur et al. (1996) reported azadirachtin (Nimbicidene) as least effective. Bt formulations, Dipel 8L and Delfin WG, and *Ha*NPV have been found to be effective for controlling *H. armigera* on chickpea (Singh et al. 1999). *Ha*NPV + endosulfan combination was reported to be superior to endosulfan or *Ha*NPV alone or neem seed kernel extract or 2% neem oil (Sivaprakasam 1998). Spark (deltamethrin 1% + triazophos 35%) and polytron C (cypermethrin 4% + profenfos 40%) have been found to be effective with or without the addition of Bt (Pal et al. 1996; Shaw et al. 1999). NPV + endosulfan have been found to be effective in sunflower (Balikai et al. 1998).

Alternate sprays of endosulfan + monocrotophos, and endosulfan + NPV have been found to be effective on chickpea (Kumawat & Jheeba 1999). Whereas Elanchezhyan (2006) reported 95 and 91% mortality of second instar larvae of *H. armigera* by synergistic effect of *B. bassiana* and *N. rileyi* with *Ha*NPV, *Bt kurstaki* and *H. indica* compared to individual effect. Mandal et al. (2007) reported more effective control of insect pests on okra by use of *Bt* + endosulfan than *Bt* alone. The treatment of deltamethrin (10g a.i./ha) proved most effective against fruit borer followed by endosulfan (1.0 lit./ha), HaNPV (400 LE/ha), neemgold (1.25 lit./ha), *Trichogramma chilonis* (450,000/ha), azadirachtin (1.5 lit./ha) whereas *Bt kurstaki* and *Bt aizawai* (@ 0.5kg/ha) existed in middle order (Sharma & Bhardwaj, 2008).

Gassmann et al. (2008) reported increase in fitness cost of *Bt* resistance due to *Steinernema riobrave* indicating that its presence in refuges may slow pest adaptation to *Bt* crops but no effect on fitness costs was detected for *Heterorhabditis bacteriophora*. Simulation modelling also supported the hypothesis that nematodes in refuges may slow resistance evolution.

In summary, pest management strategies are mostly concentrated on integrating various options such as resistant varieties, biological, mechanical and chemical control. Considerable progress has also been made in identifying source of resistance to target insect pests and incorporation of resistance traits into agronomically suitable lines. However, the advantages of host-plant resistance have not been fully realized at the farmers' level. The role of natural enemies in the management of *Helicoverpa* has been underestimated. The importance of mixed crops, crop rotations, sowing dates – though very well understood – has not been fully exploited (Rao et al. 2005).

## 2.2 EPN and their role as biological control agent

### *2.2.1 Introduction*

Nematodes, also called roundworms, are unsegmented, thread-like, vermiform and cylindrical in shape; mostly free-living but some are parasitic. Nematodes, that have parasitic association with insects, have been described from > 30 nematode families (Poinar 1990; Kaya & Stock 1997). Some of these contain species that have potential for biological control of insects: Mermithidae, Tetradonematidae (Order: Stichosomida); Allantonematidae, Phaenopsitylenchidae, Sphaerulariidae (Order: Tylenchida); Diplogasteridae, Heterorhabditidae, Steinernematidae (Order: Rhabditida) (Poinar 1979; Popiel & Hominick 1992; Lacey et al. 2001). All members of Rhabditida are bacteriophagous and many of them have phoretic associations with insects. Over time, apparently, some of them evolved as insect pathogens. Therefore, this group of beneficial nematodes is called entomopathogenic, i.e., they cause disease and reproduce in insect pests. Presently, only heterorhabditid and steinernematid are used as natural alternatives to chemical pesticides and produced commercially by various companies around the world. The microbial control potential of other nematode group is rather limited because of problems with their cultural and/or limited virulence (Koppenhöfer 2000).

Entomopathogenic nematodes belonging to genera *Steinernema* (Steinernematidae) and *Heterorhabditis* (Heterorhabditidae) are associated with symbiotic bacteria *Xenorhabdus* and *Photorhabdus* (Enterobacteriacae), respectively (Thomas & Poinar 1979; Boemare et al. 1993; Herbert & Goodrich-Blair 2007). They have been recovered from soils throughout the world (Hominick et al. 1996) and their distribution may be primarily limited by

the availability of susceptible hosts. At present, there are 83 described species of entomopathogenic nematodes in the 2 families with 64 species in *Steinernema*, 1 species in *Neosteinernema* and 18 species in *Heterorhabditis.*

Entomopathogenic nematodes received much attention during the last two decades because of their potential use as biopesticides against insect pests. EPN offer a number of advantages because they have a broad host range, kill their hosts within 48 h, can be easily produced in vivo and in vitro, can be applied with standard spray equipment, pose no threat to plants, many invertebrates, are safe to humans and other non-target organisms (Kaya & Gaugler 1993). Entomopathogenic nematodes can provide effective biological control of some important insect Orders, namely, Lepidoptera, Coleoptera, Diptera, Hemiptera, Orthoptera, Thysanoptera, etc. (Shapiro-Ilan et al. 2002b; Georgis et al. 2006; Kaya et al. 2006). However, they exhibit differences in host range, infectivity, environmental tolerance and suitability for commercial production and formulation.

*2.2.2 Biology and life cycle*

The life cycle of steinernematids and heterorhabditids include egg, four juvenile stages (separated by molts) and the adult stage. The infective stage of these nematodes is free-living, non-feeding, third stage, which is often referred to as 'dauer' (meaning enduring) or 'infective' juvenile (IJs). The dauer juvenile carries 200-2000 cells of bacterial symbiont in the anterior part of its intestine (Endo & Nickle 1994). The infective juvenile seeks out a suitable host and initiates infection. *Steinernema* infective juveniles usually invade the insect host through natural body openings such as mouth, anus and spiracle whereas, *Heterorhabditis* may also enter by direct penetration

through the cuticle. Heterorhabditid possess a dorsal tooth in anterior region of head with the help of which it gains entry into haemocoel by breaking thin cuticle of intersegmental membrane (Bedding & Molyneux 1982). Soon after reaching the haemocoel of a host, the symbiotic bacteria are released from the nematode gut (Akhurst 1982). Ciche & Ensign (2003) provided first clue to the mechanism of release of bacteria by regurgitation and exit through mouth during infection of insect larva. Dauer juvenile 'recovery' (i.e., exit from developmentally arrested third, non-feeding stage) is induced by either bacterial or insect food signals (Strauch & Ehlers 1998). During recovery, dauer juveniles release bacterial cells of their symbiont into the haemocoel of the insect, where they proliferate and produce a wide range of toxins and hydrolytic exoenzymes that are responsible for the death and bioconversion of the insect larva into a nutrient soup that is ideal for nematode growth and reproduction (Forst & Clarke 2002).

Infective juvenile becomes a feeding third-stage juvenile, feed on bacteria, metabolic by-products and molts to the fourth stage and then males and females of the first generation. After mating, the females lay eggs that hatch as J1 and molt successively to J2, J3 and J4 and then to males and females of the second generation. In *Steinernema* reproduction is amphimictic: dauer juvenile mature to become either a male or a female (Table 2.1). In *Heterorhabditis* by contrast, the dauer juveniles mature to give first generation hermaphroditic females, which give rise to a second generation of amphimictic males and females and to self-fertile hermaphrodite females and dauer juveniles (Strauch et al. 1994). Wang & Bedding (1996) studied the dynamics of population development of *H. bacteriophora* and *S. carpocapsae*

in larvae of *G. mellonella*, after injection of one or two dauer juveniles, respectively into the insect haemocoel. Under these conditions, three adult generations were produced by both nematode species (Figure 2.3). As long as abundant nutrients are available, additional adult generations develop. When the nutrients are consumed, the late J2 cease feeding, incorporate a pellet of symbiotic bacteria in the bacterial chamber (Popiel et al. 1989) and molt to J3, however, retaining the cuticle of the second stage as a sheath, and leave the cadaver in search of new hosts and may survive for several months in the absence of a suitable host.

Table 2.1. Distinguishing features of *Heterorhabditis* and *Steinernema*. †

| Phenotypic trait | *Heterorhabditis* | *Steinernema* |
|---|---|---|
| First generation adults | Hermaphroditic | Males and females |
| Bacterial location | Last 2/3 of intestine | Within specialized intestinal vesicle |
| Phylogenitic relationships‡ | Rhabditida (Rhabditidae) and Stongylida | Rhabditida (Strongyloididae, Panagrolaimidae) |
| Retention of secondary form of bacteria | No | Yes |
| Infective juveniles | With cuticular tooth<br>Excretory pore below nerve ring<br>Lateral field with 2 lines | Without cuticular tooth<br>Excretory pore above nerve ring<br>Lateral field with 6-8 lines |
| First generation males | With bursa<br>9 pairs of bursal rays (genital papillae) or a reduction of this number | Without bursa<br>10 to 14 pairs + 1 single genital papillae |

† Reproduced from Forst & Clarke (2002).
‡ After Blaxter et al. (1998).

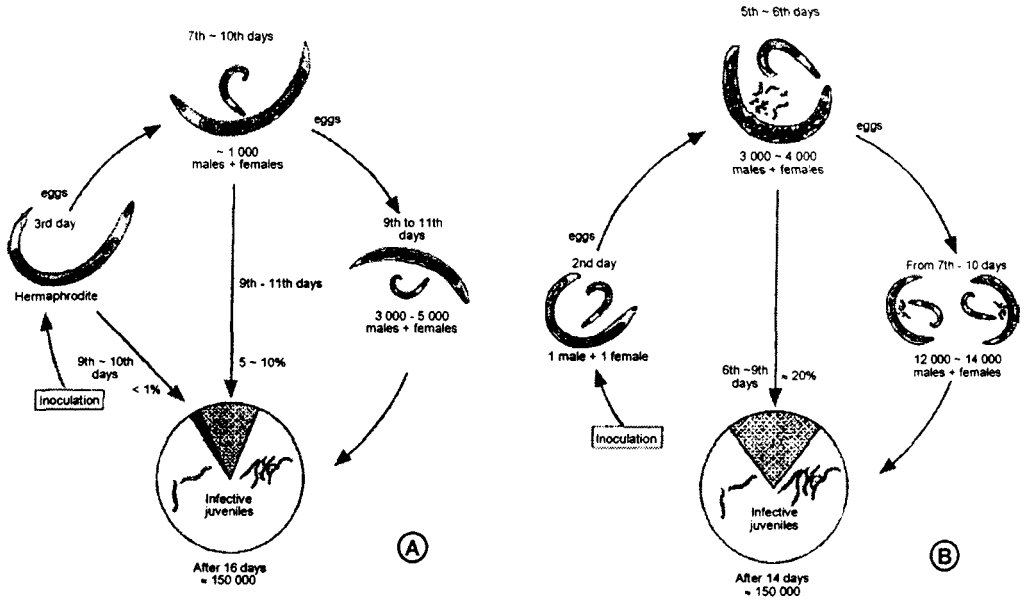



Figure 2.3. Population dynamics of A: *Heterorhabditis bacteriophora* and B: *Steinernema carpocapsae* A24 in a larva of *Galleria mellonella* after injecting one or two DJ per insect, respectively. The pie charts represent the number of DJ progeny recruited from each generation. (■): DJ progeny recruited from first generation females; (▨): DJ progeny recruited from second generation females; (▭): DJ progeny recruited from third generation females (reproduced from Wang & Bedding, 1996).

### *2.2.3 Bacteria – nematode symbiosis*

Steinernematids are associated with *Xenorhabdus* spp. and heterorhabditids are associated with *Photorhabdus* spp. (Poinar 1990; Fischer-Le Saux et al. 1999). Each nematode species is primarily associated with a single bacterial species although each bacterial species can be associated with more than one nematode (Akhurst & Boemare 1990).

*Xenorhabdus* occurs naturally in a special intestinal vesicle of *Steinernema* IJs (Bird & Akhurst 1983), while *Photorhabdus* is distributed in the fore- and mid-gut of *Heterorhabditis* IJs (Boemare et al. 1996). The relationship between nematode and bacteria is highly specific (Akhurst & Boemare 1990; Bonifassi et al. 1999). The bacteria provide nutrients to the nematodes, produce antibiotics that inhibit competing microbes, and kill the host through septicemia (Akhurst 1982; Akhurst & Boemare 1990). Although

nematodes may also contribute to host death through suppression of the immune system and toxin production (Akhurst & Boemare 1990; Simões & Rosa 1996), the most important role they play in the mutualism is serving as vectors for the bacteria. Without the nematode the bacteria cannot survive in the natural environment and are generally not pathogenic when ingested by a host (Akhurst & Boemare 1990; Morgan et al. 1997). However, Mohan et al. (2003) reported direct toxicity of *P. luminescens* to cabbage butterfly (*Pieris brassicae*) when used as a foliar spray under natural conditions.

The unique life cycle of bacteria involves the formation of mutualistic symbiosis with one host – the nematode and a vigorous pathogenic attack against a separate host – the insect. The bacteria benefit from this interaction by being protected from the competitive environment of the soil and by being transported to the nutrient-rich haemolymph of an insect (Forst & Clarke 2002). In turn, the nematode takes advantage of the pathogenic potential of the bacteria to help kill the insect host. The bacteria also supply the nutrient base for the growth and development of the nematode and suppress contamination of the insect cadaver by soil microorganisms. This remarkable co-dependent reproductive cycle is the result of a highly evolved interaction between the bacterium and the nematode.

The bacteria–nematode symbiosis can be described as a cyclic association that starts and ends with the infective juveniles in the soil. The life cycle may be divided into three stages based on milestone events in the temporal development of the bacteria and nematode (Table 2.2). First, the nematode has to migrate to look for the target host. Secondly, upon contact with host, it has to penetrate through natural body openings and/or through

the cuticle. Finally, the nematode–bacteria complex should overcome the insect immune system and be able to multiply to produce new generations of infective juveniles (Figure 2.4).

Table 2.2. Life cycle events in the bacteria – nematode symbiosis.†

| Stage | Nematode life cycle | Bacteria life cycle |
|---|---|---|
| I | Infective juvenile in the soil | Bacteria retained in nematode gut |
| | Search for insect host | |
| | Infective juvenile enters insect haemocoel | |
| II (Early) | Recovery in the haemocoel | Bacteria released into haemolymph |
| | | Production of virulence factors |
| | | Death of insect |
| II (Late) | Nematode reproduction | Bacteria in stationary phase |
| | | Production of antibiotics, exoenzymes, crystal protein |
| | | Bioconversion of insect |
| III | Development of new infective juveniles | Colonisation in the intestine of infective juveniles |

†Reproduced from Forst & Clarke (2002).

When symbiotic bacteria are released by the nematode into the insect haemolymph, the bacterial cells begin to grow and death of the insect ensues, either from toxaemia or from septicemia, depending on the sensitivity of the insect and the symbiont strain (Forst et al. 1997; Boemare & Givaudan 1998; Herbert & Goodrich-Blair 2007). Some strains of *Xenorhabdus* and *Photorhabdus* are highly virulent: injection of less than ten cells of the bacterium into the haemocoel may be sufficient to kill a susceptible insect such as *G. mellonella* or *Manduca sexta* (Poinar & Thomas 1967; Forst et al. 1997; ffrench-Constant & Bowen 1999). As the bacteria enter the stationary

phase of their growth cycle they secrete lipase(s), protease(s), several broad-spectrum antibacterial and antifungal antibiotics (Akhurst & Boemare 1990; Forst & Nealson 1996). The likely role for the degradative enzymes is to break down the insect tissues thereby providing a rich food supply for the developing nematodes. Nematode reproduction is optimal when the natural symbiont dominates the microbial flora, suggesting that the bacteria can serve as a food source and/or provide essential nutrients that are required for efficient nematode proliferation (Poinar 1990; Akhurst & Boemare 1990).

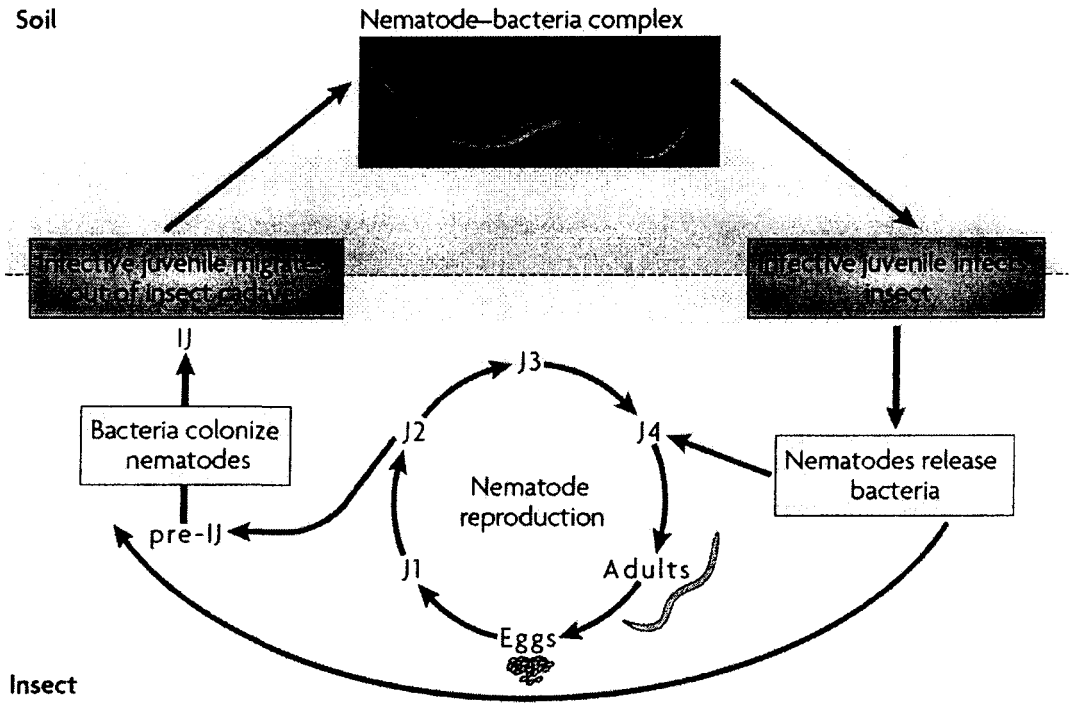



Figure 2.4. *Xenorhabdus nematophila* life cycle: The infective juvenile (IJ) nematode containing *X. nematophila* bacteria (nematode–bacteria complex) enters a susceptible insect host through natural openings that include the mouth, anus and spiracles. After entering the insect blood system, the nematode releases *X. nematophila* and develops into a fourth stage juvenile. Together, the nematode and bacteria overcome insect immunity and kill the insect. The insect cadaver is used as a nutrient source and is protected from opportunistic infection and scavenging by metabolites produced by *X. nematophila*. Within this environment, *Steinernema carpocapsae* reproduce sexually and progeny develop through four juvenile stages. Some nematodes develop into IJs after being recolonized by *X. nematophila*. The pair then exit the depleted insect carcass in search of a new host (Reproduced from Herbert & Goodrich-Blair 2007).

*2.2.4 Mass production*

The nematode-bacteria complex can be mass produced for use as biopesticides using in vivo or in vitro methods, i.e., solid or liquid fermentation (Friedman 1990; Ehlers 2001; Gaugler & Han 2002; Shapiro-Ilan & Gaulger 2002). In vivo production (culturing in insect hosts) is low technology, has low startup costs, require less capital and technical expertise and resulting nematodes quality is high, yet cost efficiency is low. In vitro solid culture, i.e., *growing the nematodes and bacteria on polyurethane foam, offers an* intermediate level of technology and costs. In vivo production and solid culture may be improved through innovations in mechanization and streamlining. In vitro liquid culture is the most cost-efficient production method but requires the largest startup capital and nematode quality may be reduced (Shapiro-Ilan & Gaugler 2002). Therefore, each approach has advantages and disadvantages relative to cost of production, capital outlay and technical expertise required, economy of scale and product quality.

In vivo production is suitable for laboratory-scale production (e.g., for generating material for field trials) and among many small nematode-producing companies (cottage industry). It is also arguably the most appropriate technology for grower cooperatives and for developing countries where labour is less expensive (Gaugler & Han 2002; Gaugler et al. 2002). Additionally, the quality of in vivo produced nematodes tends to be equal to or greater than nematodes produced with other approaches (Gaugler & Georgis 1991; Yang et al. 1997).

The most common insect host used for laboratory and commercial entomopathogenic nematode culture is the last instar of greater wax moth, *G.*

*mellonella*, because of its high susceptibility to most nematodes, wide availability (commonly used as fish bait or pet food), ease in rearing, and its ability to produce high yields (Woodring & Kaya 1988). Upto 200,000 *S. feltiae* (Dutky et al. 1964) and over 500,000 *H. bacteriophora* (Flanders et al. 1996) infective juveniles have been harvested from a single last instar *G. mellonella* larva.

In the past, steinernematids and heterorhabditids have been cultured on a variety of artificial substrates (House et al. 1965; Bedding 1981). The first successful commercial scale monoxenic culture was developed by Bedding and has come to be known as solid culture (Bedding 1981, 1984). In this method crumbed polyether polyurethane foam coated with a nutritive medium is inoculated first with symbiotic bacteria and then with nematodes, yielded up to $65.0 \times 10^6$ IJs per 500 ml flask (Bedding 1981) or $2.0 \times 10^{12}$ IJs per aerated autoclavable plastic bag (Bedding 1984). Since then, this method has been commercially used in Australia, China and United States. In a scale up model, Friedman (1990) reported that the solid culture is economically feasible up to a production level of $10.0 \times 10^{12}$ nematodes per month.

*2.2.5 Bioefficacy*

Entomopathogenic nematodes have been tested against a large number of insect pest species with results varying from poor to excellent control (Georgis et al. 2006). These nematodes have been most efficacious in habitats that provide protection from environmental extremes, especially in soil, which is their natural habitat and to a lesser extent in other cryptic habitats. For example, excellent control has been achieved against plant-boring insects because their cryptic habitats are favourable for nematode

survival and infectivity (Arthurs et al. 2004). On foliage and other exposed habitats, the infective juveniles face harsh conditions that can be only marginally remedied by adjuvants.

In the laboratory, most entomopathogenic nematode species infect a variety of insects where host contact is certain, environmental conditions are optimal, and no ecological or behavioral barriers to infection exist. Foliage feeding lepidopteran larvae are highly susceptible to infection in petri dishes, but are seldom impacted in the field, where nematodes tend to be quickly inactivated by the environmental factors (i.e., desiccation, radiation, temperature) characteristics of exposed foliage (Kaya & Gaugler 1993). However in the field, entomopathogenic nematodes attack a significantly narrower host range than in the laboratory (Georgis et al. 2006). Because these nematodes are adapted to the soil environment, the principal natural hosts are soil insects.

The efficacy of entomopathogenic nematode varies with many biological factors, including nematode species/strain, and insect species and their developmental stages (Simões & Rosa 1996). One of the factors affecting efficacy is that many soil-dwelling insects have evolved behaviors resulting in reduced host finding, attachment, or penetration by the infective juveniles. Some of the documented insect behaviors include: high defecation rate that reduces infection via the anus (scarab grubs); low $CO_2$ output or $CO_2$ released in bursts that minimize chemical cues (lepidopteran pupae and scarab grubs); formation of impenetrable cocoons or soil cells before pupation that serve as physical barriers (many lepidopterans and scarabs); walling-off nematode killed individuals that avoid or reduce contamination to other

insects in a nest (termites), and aggressive grooming/evasion behavior that reduces nematode contact (scarab grubs) (Gaugler et al. 1994; Koppenhöfer et al. 2000).

A major factor restricting the entomopathogenic nematode host range is the foraging behavior of the infective juveniles. These nematodes employ different foraging strategies to locate and infect hosts, which range from one extreme of sit-and-wait (ambush) to the other of widely foraging strategy (cruise) (Lewis 2002). The sit-and-wait strategies or ambushers (e.g., *S. carpocapsae* and *S. scapterisci*) are characterized by low motility and a tendency to stay near the soil surface. Ambushers tend not to respond to volatile and contact host cues unless presented in an appropriate sequence and efficiently infect mobile host species such as the codling moth, cutworms and mole crickets near the soil surface. At the other extreme, the widely foraging strategists or cruisers (e.g., *S. glaseri* and *H. bacteriophora*) are characterized by high motility and are distributed throughout the soil profile. Cruisers orient to volatile host cues and switch to a localized search after host contact and are well adapted to infecting sedentary hosts such as scarab and lepidopteran prepupae and pupae.

The lack of knowledge about nematode ecology has resulted in unanticipated failures to control pests in the field. For example, parasitic nematodes were found to be completely ineffective against blackflies and mosquitoes due to their inability to swim (Lewis et al. 1998). Efforts to control foliage-feeding pests with entomopathogenic nematodes were equally unsuccessful, because nematodes are highly sensitive to UV light and desiccation on a leaf surface (Lewis et al. 1998). Comparing the life histories

of nematodes and target pests can often explain such failures. Each nematode species has a unique array of characteristics, including different environmental tolerances, dispersal tendencies and foraging behaviors (Lewis et al. 1998). Increased knowledge about the factors that influence entomopathogenic nematode populations and the impacts they have in their communities will likely increase their efficacy as biological control agents.

Several factors related to the nematode's biology are critical for successful application; foremost is matching the appropriate nematode with the target pest. Proper match of the nematode to the host includes virulence, host finding and environmental tolerance (Shapiro-Ilan et al. 2006). If a nematode does not possess a high level of virulence toward the target pest, there is little hope of success. Matching the appropriate nematode host seeking strategy with the pest is also essential (Lewis et al. 1992; Lewis 2002). Environmental tolerance to desiccation or temperature may also be important in choosing the best-adapted nematode for a particular pest. Therefore, no matter how well suited an entomopathogenic nematode is to a targeted pest, the application will fail if the agent is not delivered in a manner that enables access to and infection of the host (Shapiro-Ilan et al. 2006).

To be effective, entomopathogenic nematodes must usually be applied to soil at rates of $2.5 \times 10^9$ IJs/ha or higher (Georgis et al. 1995; Shapiro-Ilan et al. 2002b). *S. carpocapsae* applied at the relatively low rate of 12.5 IJs/cm$^2$ (equivalent of $1.25 \times 10^9$ IJs/ha) reduced black cutworm, *Agrotis ipsilon* damage in field corn by more than 75%, which was as effective as or more so than the chemical insecticides tested (Levine & Oloumi-Sadeghi 1992). Whereas, some insects that are less susceptible or can be found deep below

the soil surface may require higher rates to achieve sufficient efficacy, e.g., the Diaprepes root weevil (McCoy et al. 2000; Shapiro-Ilan et al. 2002b). Generally, nematode populations can be expected to remain high enough to provide effective pest control for 2-8 weeks after application to soil under field conditions. The potential for nematode recycling and long-term pest suppression is dependent on various factors such as soil type, ground cover, host and host density and the nematode species (Kaya 1990; Shapiro-Ilan et al. 2002b).

Entomopathogenic nematodes may act in a synergistic manner along with symbiotic bacteria to provide control of lepidopteran pests that pupate in the soil. Inundative applications of *Steinernema riobrave* to control 6$^{th}$ instar larvae and pupae of *H. zea* in maize was found to be more effective under flood irrigation, causing over 90% mortality (Feaster & Steinkraus 1996). Soil moisture is regarded as an important factor in soil nematode survival and movement. Given the ability of *S. riobrave* to persist under severe environmental conditions, it may become a sustainable IPM component in flood-irrigated cropping systems. Commercially available nematode provide good suppression of larvae if they are applied to corn silk (Purcell et al. 1992). Soil surface and subsurface application of nematodes can also affect earworm populations because larvae drop to the soil for pupation (Cabanillas & Raulston 1996). This approach may have application for commercial crop protection, but the larvae must complete their development before being controlled, thereby only reducing insect pest pressure for the next cropping cycle – an approach that is unlikely to be economically feasible for farmers (Bergvinson 2005).

# 3 Pathogenicity, development and multiplication of *Steinernema masoodi* in *Helicoverpa armigera*


## ABSTRACT

The pathogenicity, development and reproductive potential of entomopathogenic nematode, *Steinernema masoodi* (Rhabditida: Steinernematidae) was investigated in *Hlicoverpa armigera* (Hübner) under laboratory conditions. The bioassays were done by noting mortality across a range of nematode concentrations, describing the temporal progression of infection, and role of the symbiotic bacteria in causing mortality by utilizing axenic nematodes. The life cycle of *S. masoodi* was completed within 4–5 days of infection at 28°C. Bacteria-free (axenic) third stage juveniles of *S. masoodi* were unable to kill *H. armigera* but become infective after acquisition of symbiotic bacteria, whereas nematode alone made no contribution towards death of insect larva. In filter paper bioassay, mean larval mortality of different

stages of *H. armigera* ranged from 43.3 to 74.8% after 72 h post-exposure of various dosage of infective juveniles (IJs). Third and fourth instar larvae (74.5 and 74.8% mortality, respectively) were more susceptible than fifth (66.2%) and second (43.3%) instar. Absolute mortality of fourth instar larvae was recorded at a dose of 100 IJs/larva whereas lowest mortality (22.7%) of second stage *H. armigera* larva was obtained at nematode concentration of 25 IJs. The highest nematodes production (mean ± SE) was obtained from last instar larva (125,460 ± 13,358) followed by fifth (64,097 ± 10,212), fourth (14,770 ± 2,619), second (10,207 ± 2,302) and third (6,765 ± 1,458) instar. Overall, mean number of 216,951 ± 15,650 IJs/g of body weight of larva was obtained.

**3.1 Introduction**

The gram pod borer (known as American bollworm), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is the most devastating insect pest of many crops of agricultural importance. It is widely distributed over the tropics and subtropics of the world (Reed & Pawar 1982; Sharma 2001; Gowda 2005). Due to increasing concern about chemical side effects, there is great interest in finding other methods of insect pests control especially utilizing biological control agents.

*Steinernema masoodi* (Ali et al. 2005a) was baited out from sandy soil of pigeonpea field at Bithoor, Kanpur, India during hot summer month when temperature was high (40-45°C). It was found infective against a number of pests of pulses, such as, *H. armigera*, *Lampides boeticus* (L.) (Lepidoptera: Lycaenidae), *Maruca vitrata* (F.) (Lepidoptera: Crambidae), *Mylabris pustulata* Thunb. (Coleoptera: Meloidae), *Rhizopertha dominica* F. (Coleoptera:

Bostrichidae) under laboratory conditions (Ahmad et al. 2005) and *Odontotermes* sp. (Isoptera, Termitidae) (Ahmad et al. 2006b).

In recent years, management of insect pests of agricultural importance through entomopathogenic nematodes has attained a new dimension with the identification of heat-tolerant species (*S. masoodi*, *S. seemae* and *S. thermophilum*) capable of parasitizing insects at temperature range of 30 to 35°C (Ganguly & Singh 2001; Ali et al. 2005ab, 2007). Several authors carried out bioefficacy studies against *Helicoverpa* larva and found *S. carpocapsae*, *S. feltiae*, *S. glaseri*, *S. masoodi*, *S. seemae*, *S. siamkayai* and *S. thermophilum* as effective in killing the larva under laboratory conditions (Glazer & Navon 1989; Karunakar et al. 1999b; Razak & Sivakumar 2001; Jothi & Mehta 2003; Ganguly & Gavas 2004; Umamaheswari et al. 2005).

A comprehensive knowledge of the nematode's life cycle and potential for reproduction is must, which is needed as a prerequisite in order to exploit their full potential under field condition. Therefore, present investigation was carried out with the objectives to study the developmental stages of *S. masoodi* and bioefficacy of *S. masoodi* against different larval instars of *H. armigera*. In vivo production potential of *S. masoodi* in *H. armigera* was also investigated.

## 3.2 Materials and methods

### *3.2.1 Nematode and insect culture*

*Steinernema masoodi* was cultured on fully grown larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae). One ml of nematode suspension (approx. 200 IJs/ml) was evenly distributed on a 9-cm filter paper in a lid of 100 × 15-mm petri dish. Ten *G. mellonella* larvae were put on the dish. The lid was covered with the inverted petri bottom and stored in BOD incubator at

28 ± 1 °C and 92% RH. After 2-3 days, nematode-infected dead larvae were removed and placed on modified White trap (Kaya & Stock 1997). Infective juveniles emerging from *G. mellonella* larvae were harvested thrice a week until production dropped (within 3 weeks). Infective juveniles were rinsed in 0.1% Hyamine® solution (methylbenzethonium chloride) and allowed to settle in a beaker. The supernatant was decanted and more sterile distilled water was added until the suspension was clear. The nematodes were stored in petri dishes (dia. 15 cm) at a concentration of 2,000 IJs/ml at water depth of 2 cm to assure sufficient aeration. The fresh culture was either used for the experiment or stored in incubator at 20°C.

The starter culture of the test insect, *H. armigera* was collected from nearby pigeonpea and chickpea fields and reared on semi-synthetic diet as described by Ali et al. (2005b) and used in the experiments. The larvae of the same age group were used in different sets of experiment with the consideration of first day of hatching as day one.

*3.2.2 Virulence test against Helicoverpa armigera*

The virulence of *S. masoodi* was tested against second to fifth instar larvae of *H. armigera* (Figure 3.1). The larvae were put in 6-well multicavity plate (dia. 35 mm) padded with rounded filter paper having one larva in each well. Different concentrations of IJs were inoculated in each well. The per cent mortality was recorded 72 h post-inoculation of IJs at 28°C and 92% RH. For each treatment, 12 individuals were treated with a specific nematode concentration. There were 24 combinations of treatments: 6 concentrations (0, 25, 50, 75, 100 and 150) × 4 instars ($2^{nd}$, $3^{rd}$, $4^{th}$ and $5^{th}$) with 3 replicates. A total of 864 *H. armigera* larvae were used in the assay.

Figure 3.1. Different lot of experiment for testing virulence of *Steinernema masoodi* against second to fifth instar larvae of *Helicoverpa armigera* in 6-well multicavity plates. Each well was padded with filter paper treated with different concentrations of *S. masoodi* and then semi-synthetic diet or chickpea foliage was given to *H. armigera* as food.

*3.2.3 Role of symbiotic bacteria in insect virulence*

Primary form of bacterial colonies from nematode was isolated as per procedure described by Kaya & Stock (1997). *S. masoodi*-infected dead *H. armigera* cadaver was surface sterilized by dipping into 95% ethanol, igniting and plunging three times into sterile distilled water (SDW). Cadaver skin was torn apart and a loop full of haemolymph was taken and streaked on MacConkey agar and incubated at 28°C. After 48 h, single colony from MacConkey was selected and streaked on NBTA for the selection of primary form of bacteria. After 2 days of incubation, appropriate colony was scrapped, transferred to nutrient broth, incubated for 48 h and used in the experiment.

One hundred *S. masoodi* giant females with developing embryos inside were collected and surface sterilized with 0.1% hyamine for 15 minutes and rinsed thrice with SDW. From them, 20 first-stage surface sterilized juveniles were picked up and put in cavity blocks each having five drops of haemolymph of *H. armigera*, which were secured tightly in moistened plastic containers. In three sets (consist of 5 cavity blocks each), 250 µl of 48 h-old bacterial suspension from nutrient broth was added while in another three sets, only SDW was added aseptically and incubated at 28°C for 24 h. Prior to virulence test, juveniles obtained were surface sterilized again as above. Thereafter, virulence test was carried out against third instar larvae of *H. armigera* and results were noted down at 48 h. Further, the dead larvae were rinsed in DSW and dissected out in ringer's solution to check the presence of nematodes penetrated and calculation was done according to Glazer & Lewis (2000).

*3.2.4 Life cycle of Steinernema masoodi*

Population of *S. masoodi* used for this study was obtained from the original stock culture. The infected hosts were obtained by placing one *H. armigera* larva in each well of 6-well multicavity plate and 200 µl SDW containing 50 IJs were added in each well and kept at 28 ± 1°C and 92% RH. At interval of 12, 18, 24, 48 and 72 h, 5 dead larvae were dissected in 25% Ringer's solution and observed for the developmental stages of the nematode. The differentiation of the developmental stages were based on the life cycle of steinernematid as described by Wouts (1980).

Observations on fecundity, time requirement for moulting and duration of each stage were made by placing 10 IJs in two drops of *H. armigera* haemolymph (by cutting its proleg and collecting oozing haemolymph) on separate concavity slides. The slides were kept on moist filter paper in a petri dish and secured tightly with Parafilm strip to retain moisture. Observations were taken under Leica DMLB research microscope.

*3.2.5 Steinernema masoodi multiplication in H. armigera*

Nematode-infected dead *H. armigera* larvae obtained from virulence test were weighed on digital weighing balance (Mettler Toledo AT 20, Sartorius) and then transferred to modified White trap and incubated at 28 ± 1°C and 92% RH and checked daily for IJs emergence after 2 days of placement. Infective juveniles were harvested every alternate day up to 3 weeks, rinsed in a beaker and quantified by serial dilution in counting dish under binocular microscope (replications: 5) and mean values were calculated.

In another set of experiment, reproductive potential of *S. masoodi* was observed by infecting only last instar larvae of *H. armigera* at nematode

concentration of approx. 150 IJs/larva. Other conditions were similar as described in section 3.2.2 (virulence test).

*3.2.6 Statistical analysis*

Mortality data presented as percentage was first corrected for control mortality following Abbott (1925) and then normalized using arc sine of the square root transformation before analysis of variance (ANOVA). The difference between means of each experiment was evaluated by Tukey's honest significant difference test (HSD) of ANOVA (SPSS 2002). Differences between them were considered significant at $P < 0.05$. The relationships between larval weight of instars and progeny production were analyzed using linear regression analysis (SPSS 2002).

**3.3 Results**

*3.3.1 Virulence test against Helicoverpa armigera*

Infective juveniles of *Steinernema masoodi* at all concentrations except control were infective to the different larval instars of *H. armigera* in laboratory bioassay (Figures 3.2 & 3.3). The interaction between nematode concentration and larval instar was highly significant ($F = 4.60$; df = 15, 46; $P < 0.001$). The larval mortality did not surpass 86% at any nematode concentration. Third and fourth instars were more susceptible than fifth and second instar larvae ($F = 53.81$; df = 3, 46; $P < 0.001$). Irrespective of nematode concentrations tested, the low mortality was recorded at lower nematode concentration which increased to 62 – 75% with increase of concentration up to 150 IJs/larva ($F = 216$; df = 5, 46; $P < 0.001$). The larval mortality of 75.0 and 72.3% at nematode concentration of 100 and 150 were on par followed by 64.5 and 62.0% mortality at 50 and 75 IJs/larva,

respectively. The lowest mortality of 49.2% was recorded at a dose of 25 IJs/larva.

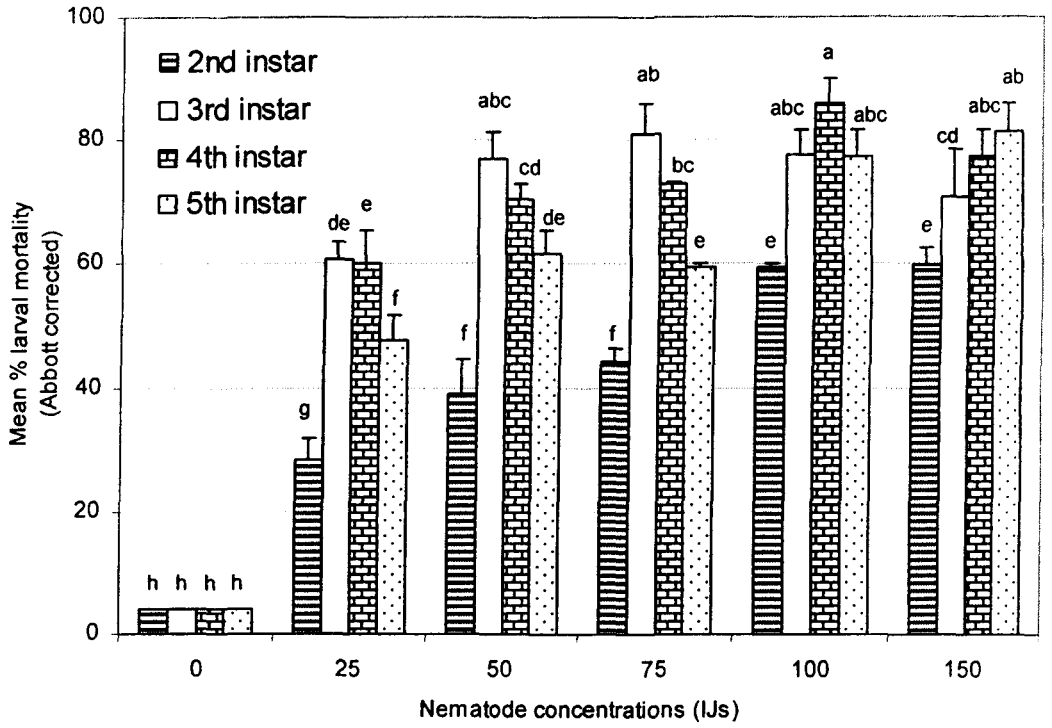



Figure 3.2. The per cent mortality of different instars of *Helicoverpa armigera* after 72 h exposure to various concentrations of *Steinernema masoodi* infective juveniles at 28 °C and 92% RH. Error bars indicate the standard errors of the means. Bars (means of 3 replicates where each replicate comprised of 12 *H. armigera* larvae) indicated with the same letter are not significantly different according to Tukey's HSD test at $P < 0.05$ (arc sine transformed values).

### *3.3.2 Role of symbiotic bacteria in insect virulency*

The test confirmed the positive role of symbiotic bacteria in causing larval mortality of *H. armigera*. Axenic juveniles did not cause larval mortality, whereas juveniles from other set, in which bacterial supplements was added, caused > 72% mortality. The average number of penetrated nematodes counted in each cadaver was 6.5 (range 4 to 9 nematodes); nematode penetrated being 10 – 22.5%.


Figure 3.3. Virulence of *Steinernema masoodi* against *Helicoverpa armigera* larvae. (A) Dead fourth instar larvae of *H. armigera* in 6-well multicavity plates. (B) Infective juveniles of *S. masoodi* emerging from *H. armigera* cadaver and multiplying over the body.

*3.3.3 Life cycle of Steinernema masoodi*

The life cycle of *S. masoodi* has an amphimictic and ovoviviparous generation. Penetration and invasion of 3rd stage juvenile (J3) into *H. armigera* took place through natural body openings, e.g., mouth, anus, and/or spiracles (Figures 3.4 & 3.5). The death of host insect was noticed 12 h onward, which turned buff or grey within 24-36 h post-infection and the colouration intensified progressively. Upon dissection, 7-12 developing nematodes were found throughout the host body. J3 released symbiotic bacteria, recovered and resumed their development, increased slightly in length, doubled in width and moulted in 24 h.

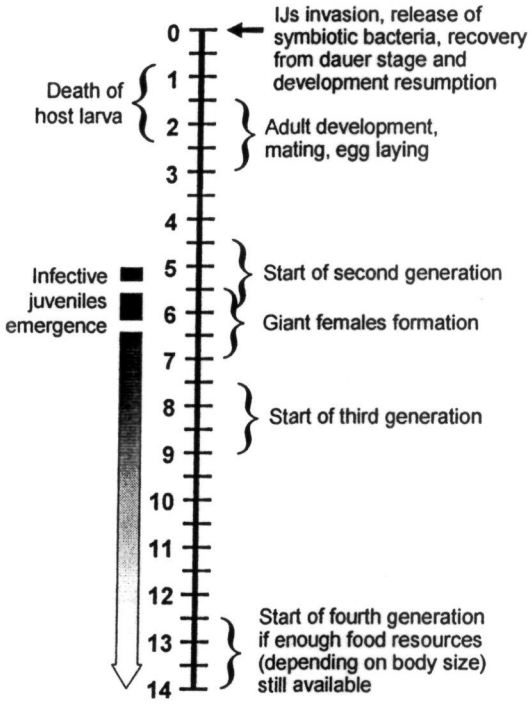
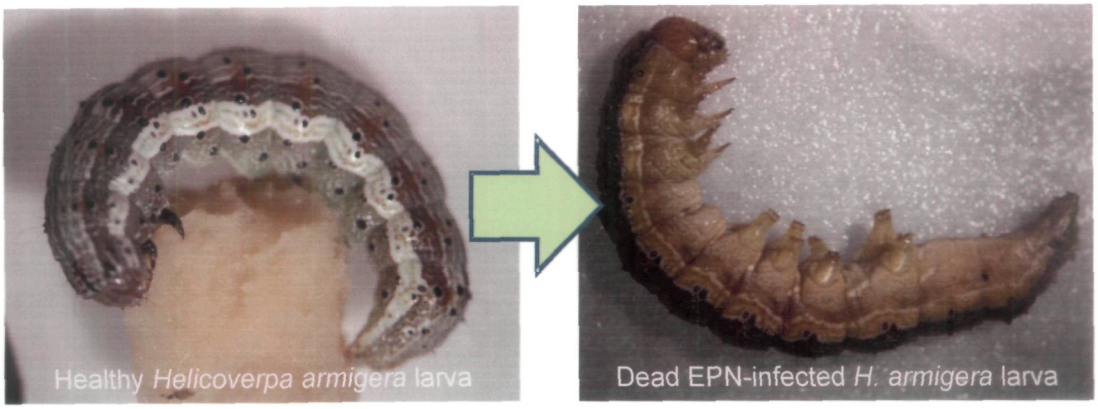
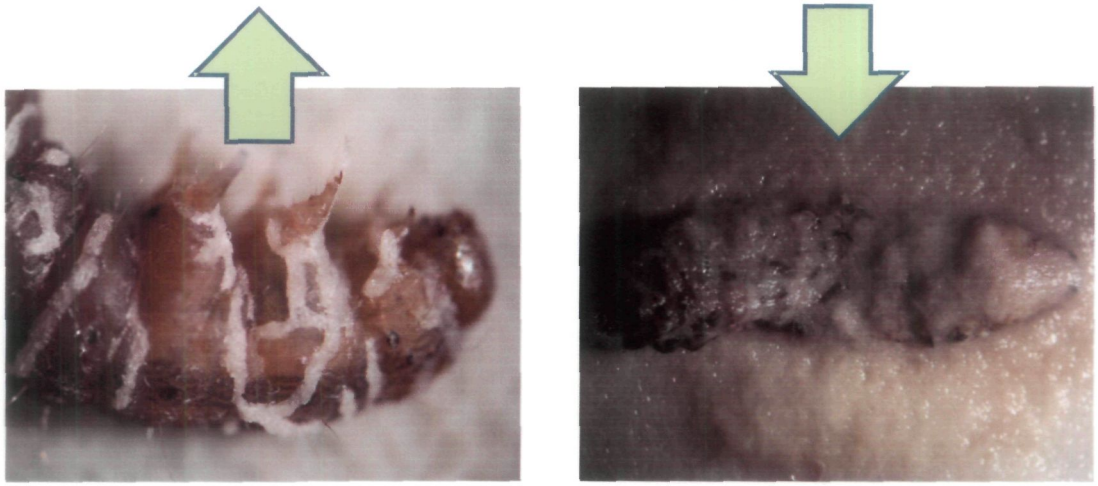



Figure 3.4. Generalized time frame (in days) of the progress of infection, development and multiplication of entomopathogenic nematode, *Steinernema masoodi* in *Helicoverpa armigera* larva (Curly bracket indicate the range of start of particular activity).



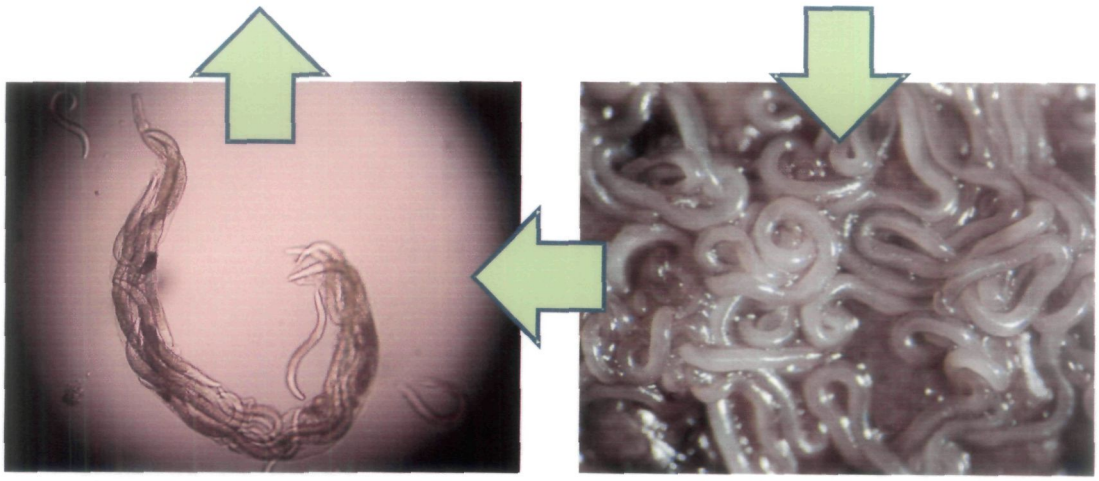

Infective juveniles (IJs) detect the potential host insect & invade via natural body openings, e.g., mouth, anus or spiracle.

IJs release symbiotic bacteria inside the body of host insect. Insect dies within 24-72 h due to septicaemia (blood poisoning).



IJs emerging out of *Helicoverpa* cadaver. This happens when food supply get exhausted, non-feeding infective stages start producing. IJs exit the cadaver in search of new host.

IJs feed on proliferating bacteria and decomposing tissues, develop into adults, mature and produce 2 or more generations in two weeks.



*Endotokia matricida* - a process of IJs development causing maternal death under condition of nutritive depletion.

IJs developed into giant adults.

Fig. 3.1 Life cycle and mode of action of *Steinernema masoodi* in *Helicoverpa armigera* larva.

In the next 24 h, J4 (characterized by noticeable reproductive organs) developed into male or female in the ratio 1 : 3.4. Males were shorter in body length and moved more rapidly. The measurement (in mm) in the form mean ± SD (range; sample size) for first generation male, female and giant form adults were 0.76 ± 0.15 (0.652 to 0.839; *n* = 9), 1.53 ± 0.27 (1.011 – 1.871; *n* = 12) and 5.99 ± 0.55 (5.227 – 6.750; *n* = 5), respectively. Female laid approx. 737 ± 179 (range 583 to 992; *n* = 10) oval-shaped eggs (62 μm × 26.5 μm) in its life span. Embryonic development was observed within few hours of egg laying and hatching of first stage juvenile ($J_1$) took place in about 10 h. With availability of food in degrading host, more eggs were laid for 3 days when nematode population density was low in host. However, those eggs which remained inside the uterus after the period of egg laying, developed inside the body of female into next stage (J2) by feeding maternal tissues and resulted in death – a phenomenon known as *endotokia matricida* (Johnigk & Ehlers 1999). An average of 34 ± 6.5 (range 22 to 45; *n* = 10) developing J2 juveniles emerged per female by this means. Whether eggs were laid or retained within the body, they developed rapidly into males or females of the second generation. Sex ratio of male to female was 1 : 9.2 ± 4.6. The life cycle of second generation was completed in about 72 h, which was shorter than the entire first generation cycle. When the quantity of food decreased in the host larvae, J2 (developmental stage which can develop into third developmental or infective stage) developed into infective juveniles by incorporating a pellet of bacteria in its bacterial chamber. They, then, moulted to J3 infective stages, retaining the cuticle of the second stage juvenile as a protective sheath and started moving out of the degrading host body in nearby vicinity.

*3.3.4 Steinernema masoodi multiplication in H. armigera*

A modest to strong correlation existed between larval weight and *S. masoodi* infective juveniles production (Figures 3.6 & 3.7; coefficient of determination, $R^2$, varied from 0.532 to 0.864). The linear relationship is described by the equation $y = a + bx$ where $y$ is dependent variable (progeny production (yield)) and can be expressed in terms of a constant ($a$) and a slope ($b$) times the independent variable $x$ (larval weight (wt)). A strong correlation existed for second and fourth instar larvae (Figure 3.6A; $F = 15.17$; df = 1, 5; $P = 0.011$ and Figure 3.6C; $F = 35.54$; df = 1, 8; $P < 0.001$, respectively). Whereas, modest correlation existed for third and fifth instar larvae (Figure 3.6B; $F = 6.81$; df = 1, 6; $P = 0.04$ and Figure 3.6D; $F = 10.47$; df = 1, 8; $P = 0.012$, respectively). A strong correlation was also found for the last instar larvae (Figure 3.7; $F = 177.81$; df = 1, 28; $P < 0.001$).

**3.4 Discussion**

*Steinernema masoodi* was found to be pathogenic to different larval instars of *H. armigera* at all nematode concentrations, however, the dose–mortality relationship was not linear. Comparatively higher infectivity was observed to third and fourth instars (61.9% mortality) than to fifth (55.3%) and second (39.1%) instar larvae. Decrease in pathogenicity with age of larvae of *Pseudaletia unipuncta* Haworth (Lepidoptera: Noctuidae) to *S. carpocapsae* was also reported by Medeiros et al. (2000).

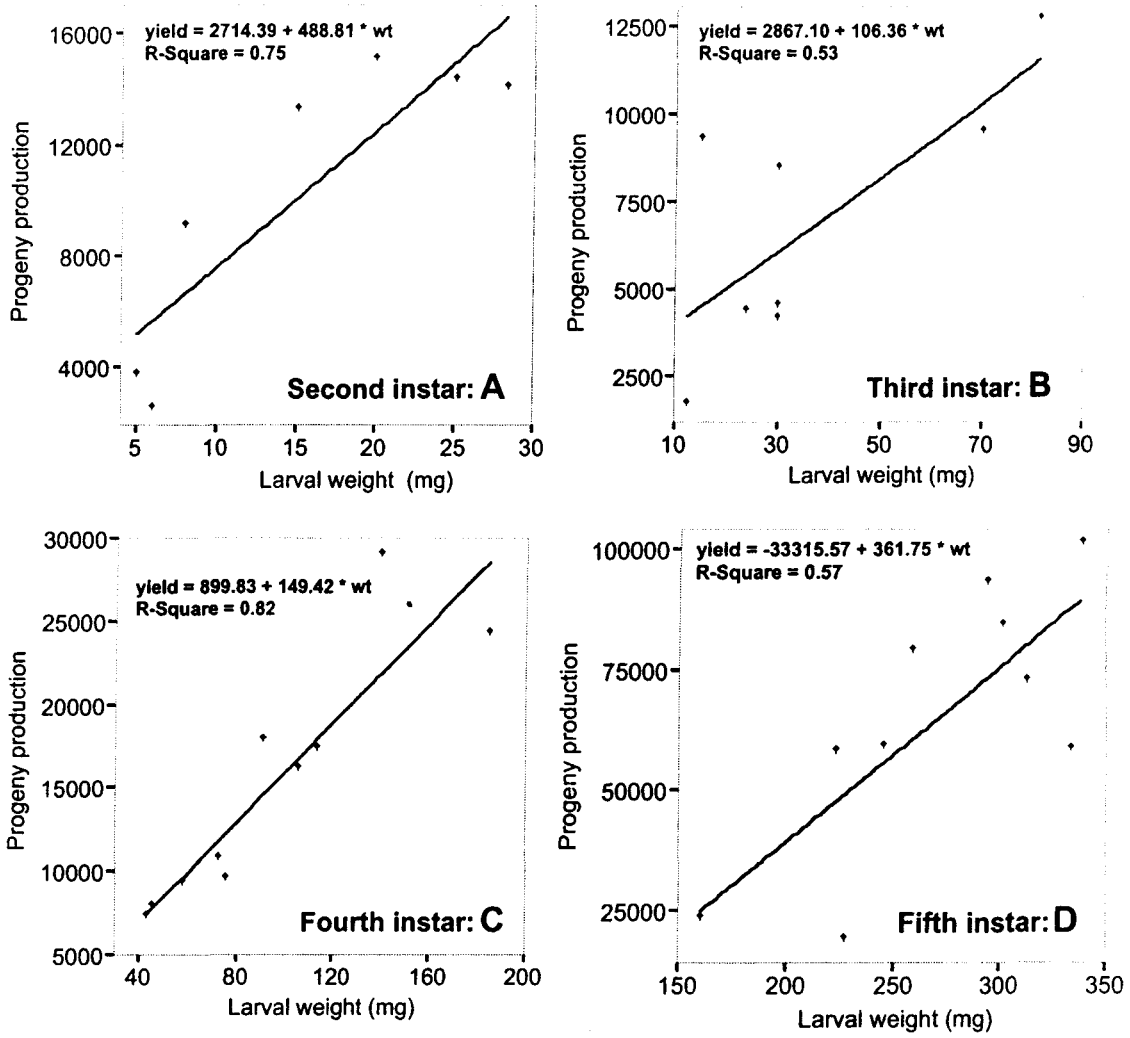



Figure 3.6. Relationship between *Steinernema masoodi* progeny production and *Helicoverpa armigera* larval weight. The number of solid filled circles in panels are 7, 8, 10 and 10, which is the number of modified White traps used to harvest the emerging nematodes; one solid filled circle represent average yield per larva of same age group ($n$ = 216).

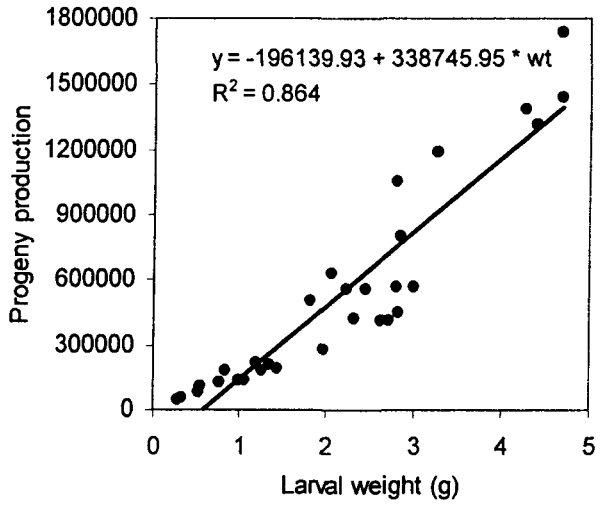



Figure 3.7. Relationship between *S. masoodi* progeny production and *H. armigera* larval weight. Solid filled circle represent average progeny produced per larva ($n$ = 326).

The dose–mortality response indicated that *H. armigera* was highly susceptible to *S. masoodi* with respect to different larval stages. In the present study, highest mortality of 85.9% was observed at nematode concentration of 100 IJs per larva of fourth instar. The pathogenicity of entomopathogenic nematodes has been found to vary with the species of insect, nematode and stages of host insect (Karunakar et al. 1999b; Rosa et al. 2002). Jothi & Mehta (2003) reported complete mortality of fourth instar larvae of *H. armigera* by 80 IJs of *S. feltiae.* Whereas, *S. riobrave* and *S. abbasi* were reported to cause 91.7 and 58.3% mortality of fourth instar larvae of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) at 200 IJs/larva (Abbas & Saleh 1998). Absolute mortality of last instar larvae of *H. armigera* was achieved with 200 IJs of *S. feltiae* strain All (Glazer & Navon 1989) and 40 IJs of *S. glaseri* or *S. feltiae* (Karunakar et al. 1999b).

The developmental stage of the insect plays an important role in susceptibility to the nematode. A variety of reasons could explain the differences, including size, immune response and host behaviour. The portals of entry for nematodes may be smaller in the younger instars (Jackson & Brooks 1995) and smaller instars may be less attractive in terms of host cues such as $CO_2$ or kairomones (Kaya 1985). However, in older insect larva, nematodes may get crushed by insect's mandibles (Gaugler & Molloy 1981) or frequent defecation may expel nematodes entering through anus (Dowds & Peters 2002). After invasion, older larvae may also become less susceptible if nematodes fail evasion of host defences and are not able to overcome the insect's immune system (Simões & Rosa 1996).

Bacteria-free (axenic) third stage juveniles of *S. masoodi* were unable to kill *H. armigera* but they become infective after acquisition of symbiotic bacteria showing the nutritive dependence of nematode on its associated bacteria. Nematode was found not contributing towards death of insect. Akhurst (1986) also observed that *S. glaseri* was not able to kill *G. mellonella* without its symbiotic bacteria. However, axenic *S. carpocapsae* and *S. feltiae* killed *G. mellonella* larvae with the possible involvement of entomotoxins (Burman 1982; Ehlers et al. 1997; Han & Ehlers 2000).

The development and life cycle of *S. masoodi* resembles with other species of steinernematid with respect to developmental stages, viz., egg, four juvenile stages and adults (male and female) (Wouts 1980; Adams & Nguyen 2002). The third stage IJs entered into *H. armigera* larva and delivered associated bacteria contained in the anterior part of the intestine, killing the larva within 24-72 h and completed two to three generations in 14 days. Close microscopic observation revealed juvenile and adult stages of *S. masoodi* coming out of its mouth, anus, spiracles and intersegmental integument from fourth/fifth day of infection and covered partially or whole body with the advancement of reproductive cycle. One striking feature noticed in *S. masoodi* life cycle was that it completed 3 generations within 2 weeks and then proceeded to next generation provided sufficient nutrients were still present in the form of degrading body tissues (especially in older instars).

In present study, *S. masoodi* completed its development at 28°C within 4 – 5 days after initial infection. *S. thermophilum* has been reported to develop, reproduce, and produce progeny emerging from insect cadavers within 4 – 6

days (Ganguly & Gavas 2004) whereas *S. abbasi* and *S. riobrave* were reported to complete life cycle in 3½ to 4½ and 5 days (Elawad et al. 1999).

The third stage IJs produced from the second generation adult started emerging en masse from *H. armigera* cadaver 4 days post-inoculation and approximately 84% IJs were produced within 10-12 days of initial IJs invasion into the host. While progeny production dropped after 14 days yielding 11% population and remaining were obtained till the nutrient of cadaver exhausted. Wang & Bedding (1996) reported *S. carpocapsae* populations reached their maximum levels (60%) by the 10th day in *G. mellonella* whereas final populations containing only IJs were obtained at the end of third week.

In the present study, average production of *S. masoodi* obtained from last instar larva of *H. armigera* was 124,280. However, Razak & Sivakumar (2001) recorded *H. armigera* as a susceptible host of *S. feltiae* DD–136 strain and on an average 187,746 IJs were harvested per larva and 342,600 IJs/g of body weight. In our studies, comparatively low production could be due to the difference in host size, host suitability and nematode species as reported on the variability of host quality have been documented by Barbercheck (1993).

The intercept of the regression equation for fifth and final instar larvae on the y–axis were negative (a = – 33315.57 and – 196139.93, respectively), which indicates that a little amount of body weight of large-sized larvae do not contribute towards the progeny production. On average, 216,951 ± 15,650 IJs per g of body weight of larva was obtained. Comparatively 298,700 *S. feltiae* per g body weight of *H. armigera* was obtained by Karunakar et al. (1999b).

It is inferred from the studies that the newly described species of entomopathogenic nematode, *S. masoodi* is pathogenic to *H. armigera*. The

bioefficacy studies, life cycle and progeny production per unit body weight give an insight into correct selection of stage of insect for mass production of entomopathogenic nematode for laboratory and field trials. This information was hitherto not studied for *H. armigera*. The study also opens the avenues to work on utilization of this potent biocontrol agent in field trials against various lepidopteran and other pests infesting field crops, which is mostly chemical dependence and organic farming is the cry of the day.

# 4 Susceptibility of three lepidopteran pests to five *Steinernema* species and production of these nematodes


## ABSTRACT

The investigation was conducted in pots to access the susceptibility of three lepidopteran pests, namely, *Helicoverpa armigera*, *Galleria mellonella*, and *Corcyra cephalonica*, to two recently described species of entomopathogenic nematodes, *Steinernema masoodi*, *S. seemae*, and three indigenous *S. carpocapsae*, *S. glaseri* and *S. thermophilum*. The suitability of these lepidopterans for the in vivo production of these nematodes was also evaluated. Among the five nematodes species, *S. masoodi*, *S. seemae* and *S. carpocapsae* were found most pathogenic to *C. cephalonica* (causing mortality within 24 h) followed by *H. armigera* (36, 38 and 48 h, respectively) and *G. mellonella* (30, 36 and 48 h, respectively). The other species, viz., *S. glaseri* and *S. thermophilum* was least pathogenic, which killed the larvae of

*C. cephalonica* in 29 and 36 h, respectively, *G. mellonella* in 48 h, and *H. armigera* in 38 and 56 h, respectively. *Galleria mellonella* was found to be the most suitable host with regards to production of *S. seemae* infective juveniles (IJs), which yielded higher progeny than *S. carpocapsae*. *Helicoverpa armigera* was the next best suitable alternate host, which produced maximum IJs in case of *S. seemae* followed by *S. masoodi*, *S. carpocapsae*, *S. glaseri* and *S. thermophilum*. *Corcyra cephalonica* was the least suitable host.

**4.1 Introduction**

Over dependence on pesticides is still widespread in spite of associated problems such as the development of insect resistance to insecticides, pest resurgence and outbreak of secondary pests and other socio-economic problems. Therefore, there is a need to identify suitable alternative methods for the management of insect pests. Integrated pest management (IPM) applies multiple methods to suppress pest populations, thereby reducing dependence on conventional insecticides, which can have unintended harmful consequences for the environment and human health (Dent 2000). Among these, entomopathogenic nematodes are emerging as a potent candidate that can be used as biopesticide for the management of lepidopteran pests (Poinar 1990; Cabanillas et al. 1994).

Entomopathogenic nematodes can be mass produced in vivo where the insect serves as a small biological reactor. *Galleria mellonella* has been widely used for nematodes mass production, while other insect like *Chilo sacchariphagus indicus* (Kapur) have also been used to study the infectivity and multiplication of *Steinernema feltiae*, *S. glaseri* and *Heterorhabditis bacteriophora* (Karunakar et al. 1992).

This study was undertaken to broaden the list of tested insect pests to which these entomopathogenic nematodes are pathogenic. The comparative virulence of *Steinernema masoodi*, *S. seemae*, *S. carpocapsae*, *S. glaseri* and *S. thermophilum* (Rhabditida: Steinernematidae) and their mass production were studied against final instar larvae of lepidopteran pests, *Helicoverpa armigera* (Lepidoptera: Noctuidae), *Galleria mellonella* (Lepidoptera: Pyralidae) and *Corcyra cephalonica* (Lepidoptera: Pyralidae).

**4.2 Materials and methods**

Five species of entomopathogenic nematodes, namely, *Steinernema masoodi*, *S. seemae*, *S. carpocapsae*, *S. glaseri* and *S. thermophilum*, were tested against final instar larva of *G. mellonella* (reared on artificial diet) and field collected larvae of *H. armigera* and *C. cephalonica* (reared on broken coarse maize grains). One-hundred gram of sterilized soil was put in each earthen pot (capacity 150 ml) and moisture maintained (13%) according to the field capacity of the soil. The freshly emerged infective juveniles (IJs) of each species of *Steinernema*, multiplied on *G. mellonella* larvae, were harvested and surface sterilized with 0.1% Hyamine solution. Approxmilately 1,000 IJs from the suspension was inoculated with the help of micropippete in sterilized soil and a single final instar larva of the test insect was introduced in earthen pot ($n$ = 15). The experiments were conducted in BOD at 28 ± 1 °C and replicated along with control. Observations were made at 6 h intervals.

Nematode-infected dead larvae of test insects were removed from earthen pots, kept on modified White trap for nematode emergence, which were collected daily up to 2 weeks till the IJs emergence stopped from insect cadavers. The collected nematodes were counted three times in a Syracuse

counting dish under Leica MS 5 stereoscopic binocular microscope and mean values were calculated.

Mortality data was normalized using arcsine of the square root transformation before analysis of variance (ANOVA). The difference between means was evaluated by Tukey's HSD test of ANOVA (SPSS 2002). Differences between them were considered significant at $P < 0.05$.

**4.3 Results and discussion**

Among the five species of entomopathogenic nematodes tested, *S. masoodi*, *S. seemae* and *S. carpocapsae* were found to be most virulent to *C. cephalonica* causing mortality at 24 h (Figure 4.1). Other species, viz. *S. glaseri* and *S. thermophilum* were less pathogenic to *C. cephalonica* (29 and 36 h post infection, respectively). The mortality of *H. armigera* by *S. seemae* and *S. carpocapsae* were recorded at 36 h followed by *S. glaseri* (38 h), *S. masoodi* (48 h); whereas *S. thermophilum* was least pathogenic causing mortality at 56 h. On the other hand, *S. seemae* and *S. masoodi* killed the larva of *G. mellonella* at 30 and 36 h, respectively, followed by mortality of 48 h recorded by *S. carpocapsae*, *S. glaseri* and *S. thermophilum* (Figure 4.1A).

With respect to nematode yield per cadaver, *G. mellonella* was the most suitable host from which highest production of *S. seemae* ($2.11 \times 10^5$ IJs/larva) followed by *S. carpocapsae* ($1.92 \times 10^5$ IJs/larva) (Figure 4.1B). Whereas low progeny ($0.40 \times 10^5$ IJs/larva) of *S. thermophilum* was recovered from *G. mellonella* larva. *Helicoverpa armigera* was the next suitable alternate host in which *S. seemae* yielded $1.53 \times 10^5$ IJs/larva followed by *S. masoodi* ($1.26 \times 10^5$ IJs/larva), *S. carpocapsae* ($1.17 \times 10^5$

IJs/larva), *S. glaseri* (0.92 × $10^5$ IJs/larva) and *S. thermophilum* (0.46 × $10^5$ IJs/larva).

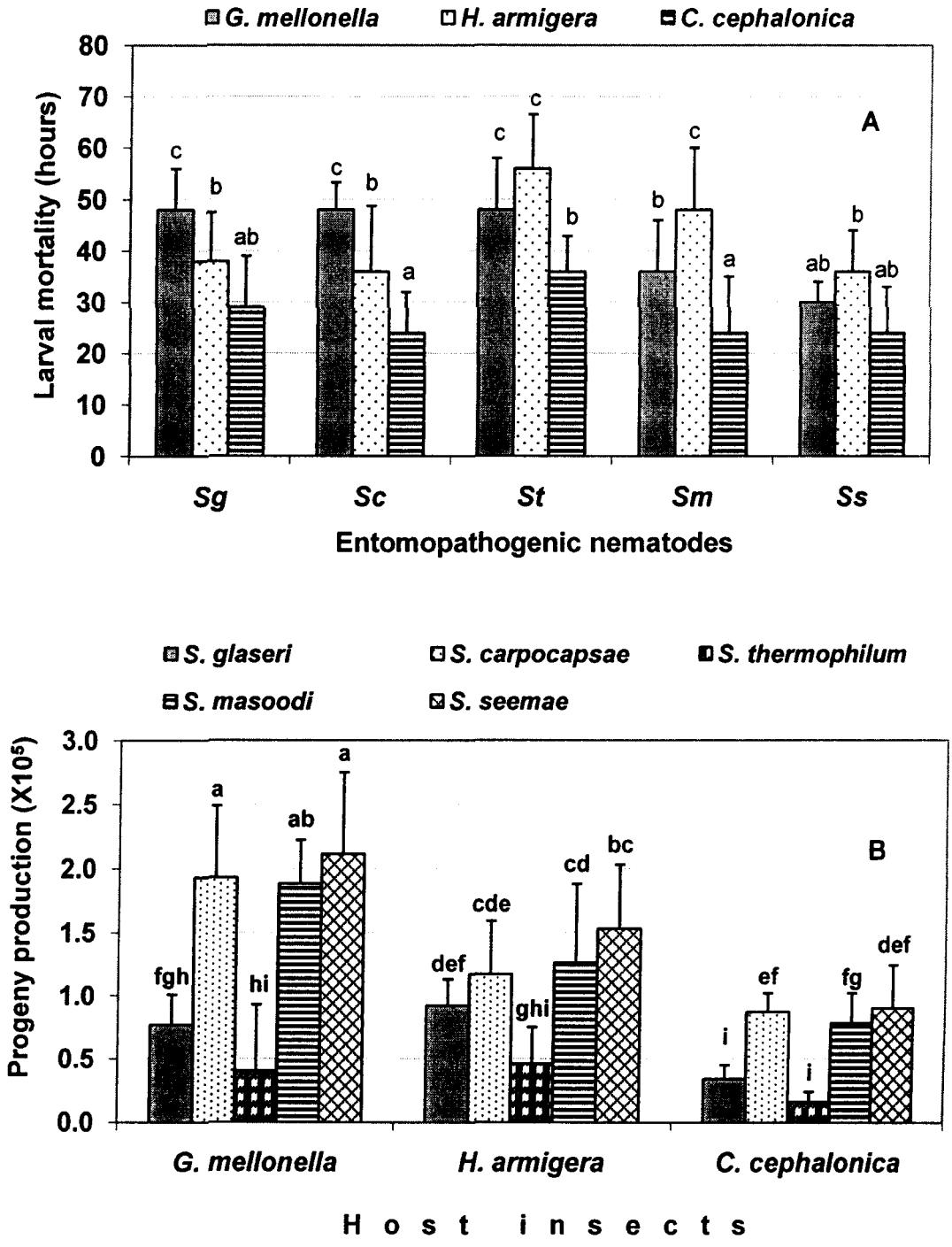



Figure 4.1. (A) Mortality of three lepidopteran pests by five entomopathogenic nematodes (EPN). Sg, *S. glaseri*; Sc, *S. carpocapsae*; St, *S. thermophilum*; Sm, *S. masoodi*; Ss, *S. seemae*. (B) In vivo mass production of five EPN on three lepidopteran species. Different letters among means were considered significant at $P < 0.05$. No larval mortality was recorded in control, therefore, control bars were exclude from the figure.

Rice moth, *C. cephalonica*, was the least suitable host from which 0.90 × $10^5$ IJs of *S. seemae* and 0.87 × $10^5$ IJs of *S. carpocapsae* per larva was obtained. The least production of *S. glaseri* (0.34 × $10^5$ IJs/larva) followed by *S. thermophilum* (0.16 × $10^5$ IJs/larva) was recorded from *C. cephalonica* (Figure 4.1B).

The least multiplication of *S. glaseri* was observed in *C. cephalonica*, which agrees with the studies of Karunakar et al. (1999b). *S. masoodi* was capable of killing the larva of *G. mellonella* within 36 h and yield of IJs on *H. armigera* was the second highest. Progeny production of *S. masoodi* and *S. carpocapsae* were on par with respect to the test insects.

It can be concluded that *S. seemae* was more pathogenic than other studied species to the larvae of three lepidopteran pests. The most suitable host for multiplication were *G. mellonella* and *H. armigera*, and these insects can be selected as the alternate host for in vivo production of nematodes under laboratory conditions. *S. seemae* is also promising, being the most pathogenic, giving highest IJs yield when infected to *G. mellonella*.

# 5 Effect of temperature on survival of infective juveniles of *Steinernema seemae*, *S. masoodi* and *S. carpocapsae* and their infectivity to prepupa of *Helicoverpa armigera*


## ABSTRACT

The survival and infectivity of three indigenous entomopathogenic nematodes, *Steinernema seemae*, *S. masoodi* and *S. carpocapsae* at different temperatures (15, 20, 25, 30, 35, 40 and 45°C) were studied against prepupa of *Helicoverpa armigera* (Hübner). The survival percentage of nematodes decreased with increase in temperature. However, 47% of the populations were able to survive and tolerate the sub-lethal temperature (45°C) treatment for 6 h. Out of the populations that survived, 43% infectivity was observed in *H. armigera* prepupa. The survival did not differ at 25 and 30°C, rather their activity was found optimum. The next temperature regimes of higher survival

was 20 and 35°C (66 and 60% survival, respectively) followed by 15°C (53%), 40°C (51%) and 45°C (47%). Overall, *S. seemae* and *S. carpocapsae* had better survival (65 and 63%, respectively) than *S. masoodi* (57%). These heat-tolerant isolates could play a vital role in the management of susceptible stages of *H. armigera* at high temperature regimes and for the management of other insect pests of agricultural importance, which pupate in the soil.

**5.1 Introduction**

Entomopathogenic nematodes have been recovered from soils of a wide variety of climatic regions in India (Karunakar et al. 1999a; Ganguly & Singh 2001; Hussaini et al. 2004). Studies on *Steinernema abbasi*, *S. tami*, *S. carpocapsae*, *S. feltiae*, *S. glaseri* and *S. thermophilum* have revealed that optimum temperature and moisture requirement for their infectivity and survival vary from species to species (Karunakar et al. 1999a; Ganguly & Singh 2001; Ganguly & Gavas 2004; Hussaini et al. 2004) depending on the nematode's climatic origin from where these are isolated.

Cooler temperatures have not been detrimental to nematode survival (Kaya 1990). *S. anomali*, *S. feltiae*, and *Heterorhabditis bacteriophora* were all found to be freezing tolerant. The lower lethal temperatures were –22, –19 and –14 °C for *S. feltiae*, *H. bacteriophora* and *S. anomali*, respectively (Brown & Gaugler 1996) but *S. riobrave*, *S. carpocapsae* and *S. glaseri* survived prolonged exposure to freezing at –4 °C (Brown & Gaugler 1998). On the upper limit, temperatures above 30°C tend to inhibit nematode's development in a host (Milstead 1981). For example, Grewal et al. (1994) reported reduced virulence and reproductivity at above 30°C. However,

temperature above 35°C over an extended period of time is detrimental to infective juveniles (Schmiege 1963).

Mortality and/or reduced infectivity of juveniles under field condition is one of the most important factors restricting their application in sub-tropical ecosystems where temperatures can be very high. The present investigation was undertaken to determine the upper limit of heat tolerance and infectivity of *S. seemae*, *S. masoodi* and *S. carpocapsae* against prepupal stage of *Helicoverpa armigera*, which after completing its larval stage undergoes into the soil for pupation.

## 5.2 Materials and methods

### *5.2.1 Nematodes and insect culture*

*Steinernema seemae* and *S. masoodi* were obtained by baiting soil samples brought from chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* (L.) Millsp.) growing regions of Hamirpur and Kanpur (Bithoor locality) districts of Uttar Pradesh, India (Ali et al. 2005a). *S. carpocapsae* was procured from Nematology laboratory, Project Directorate of Biological Control, Bangalore, India, which was originally isolated from Kanpur. All these isolates were cultured on fully grown larvae of *Galleria mellonella* as per the procedure described by Woodring & Kaya (1988). Emerged infective juveniles (IJs) were surface sterilized in 0.1% Hyamine solution and stored in distilled water in tissue culture flasks. Up to one-week-old cultures were used in the experiments. The test insect, *H. armigera* larvae were collected from pigeonpea/chickpea fields and reared on semi-synthetic diet as described by Armes et al. (1992).

*5.2.2 Heat tolerance assay*

The survival of *S. seemae*, *S. masoodi* and *S. carpocapsae* were assessed under seven controlled temperatures regimes of 15, 20, 25, 30, 35, 40 and 45°C. A known quantity of 10,000 IJs of each nematode species was placed in 100 g sterilized sandy loam soil maintaining 9% moisture level (w/v) in a small earthen pot (10 × 7 cm). Heat-treatment to nematode was given in BOD incubator for 6 h. After the exposed period of temperature treatment, pots were taken out and distilled water was added to maintain the moisture level in the soil. There were three replicates in each treatment.

The soil from each of the earthen pots was sieved and IJs were extracted with the help of modified Cobb sieving and decantation (Cobb 1918) and Baermann's funnel methods (Baermann 1917). The surviving IJs in soil were counted under Leica MS 5 stereoscopic binocular microscope in a Syracuse counting dish and mean values ($n$ = 3) were worked out.

Virulence was assessed by the ability of nematode to kill prepupa of *H. armigera*. Bioassay was conducted by releasing heat-treated nematodes (1,000 IJs) in earthen pot, introducing one *H. armigera* prepupa along with several pieces of semi-synthetic diet, covered with muslin cloth and left for three days at 30 ± 1°C. Observations were taken 24 h interval upto one week to check the mortality of prepupa. There were four sub-treatments in each treatment along with control (one replicate contained a group of 10 individual prepupae). In total, 840 *H. armigera* [7 temperatures × 4 nematodes (3 nematodes + control) × 3 replications × 10 prepupae] were utilized in the experiment.

*5.2.3 Statistical analyses*

Mortality data presented as percentage was first corrected for control mortality following Abbott (1925) and then normalized using arcsine of the square root transformation before analysis of variance (ANOVA). The difference between means of both the experiments were evaluated by Tukey's honest significant difference test (HSD) of ANOVA (SPSS 2002). Differences between them were considered significant at $P < 0.05$.

**5.3 Results and discussion**

Infective juveniles of indigenous populations of *S. seemae*, *S. masoodi* and *S. carpocapsae* were able to survive at all the tested temperatures. The temperature regime of 20 to 35°C better suited as nematode survival ranged from 60.2 to 80.3% (Figure 5.1A). The highest survival of 80.3% was recorded at 25°C followed by 75.9% at 30°C which was on par. The next temperature regimes of higher survival was 20 and 35°C (65.7 and 60.2% survival, respectively) followed by 15°C (53.3%), 40°C (50.5%) and 45°C (46.6%). Overall, *S. seemae* and *S. carpocapsae* had better survival (65.2 and 63.1%, respectively) than *S. masoodi* (57.0%).

The effect of temperatures on nematode performance was reported to vary with nematode species and strains (Kaya 1990; Grewal et al. 1994). Generally, IJs become sluggish at lower temperature (<10 – 15°C) but inactivated at higher temperatures (>30 – 40°C). Extended exposure to temperature below 0°C and above 40°C is lethal to most entomopathogenic nematode species, but the effect also depends on the duration of exposure. In the soil environment, IJs are normally buffered from temperature extremes or usually have enough time to move down into deeper soil layers where the

buffering effect is stronger. The studies conducted by Grewal et al. (1994) and Campbell et al. (1995) indicate that, in nature, nematode populations migrate upward or downward throughout the cross-section of their immediate environment in response to adverse temperature change.

Highest infectivity of prepupa of *H. armigera* was observed by *S. seemae* (68.9%) followed by *S. masoodi* (58.6%) and *S. carpocapsae* (58.2%) at all tested temperatures (Figure 5.1B). Infectivity of nematodes was affected at lower and upper limits of temperature. However, optimum infectivity of 70.0% by all nematodes was recorded at 25°C followed by 30°C (57.5%). Ishibashi et al. (1981) reported that DD-136 caused 100% mortality of 5th instar larvae of *Spodoptera litura* (Fab.) at 25–30°C in two days and it was 90% and 75% at 20 and 15°C after five days, respectively. The optimal infection, reproduction and multiplication took place between 25 and 28°C (Kaya 1977; Molyneux 1986). *S. feltiae*, when tested against pre-pupa, pupa and adult of *S. litura* at 10000, 1000 and 100 nematodes, resulted in complete mortality of the insect. The pupa was less susceptible than the pre-pupa or adult (Narayanan & Gopalakrishnan 1987).

Though both the species, *S. seemae* and *S. masoodi*, have been isolated from region of high temperature zone, but the former seems to tolerate it better than the later nematode, so much so that at 45°C, its infectivity was 46.7% against 35.9% in the later and *S. carpocapsae*.

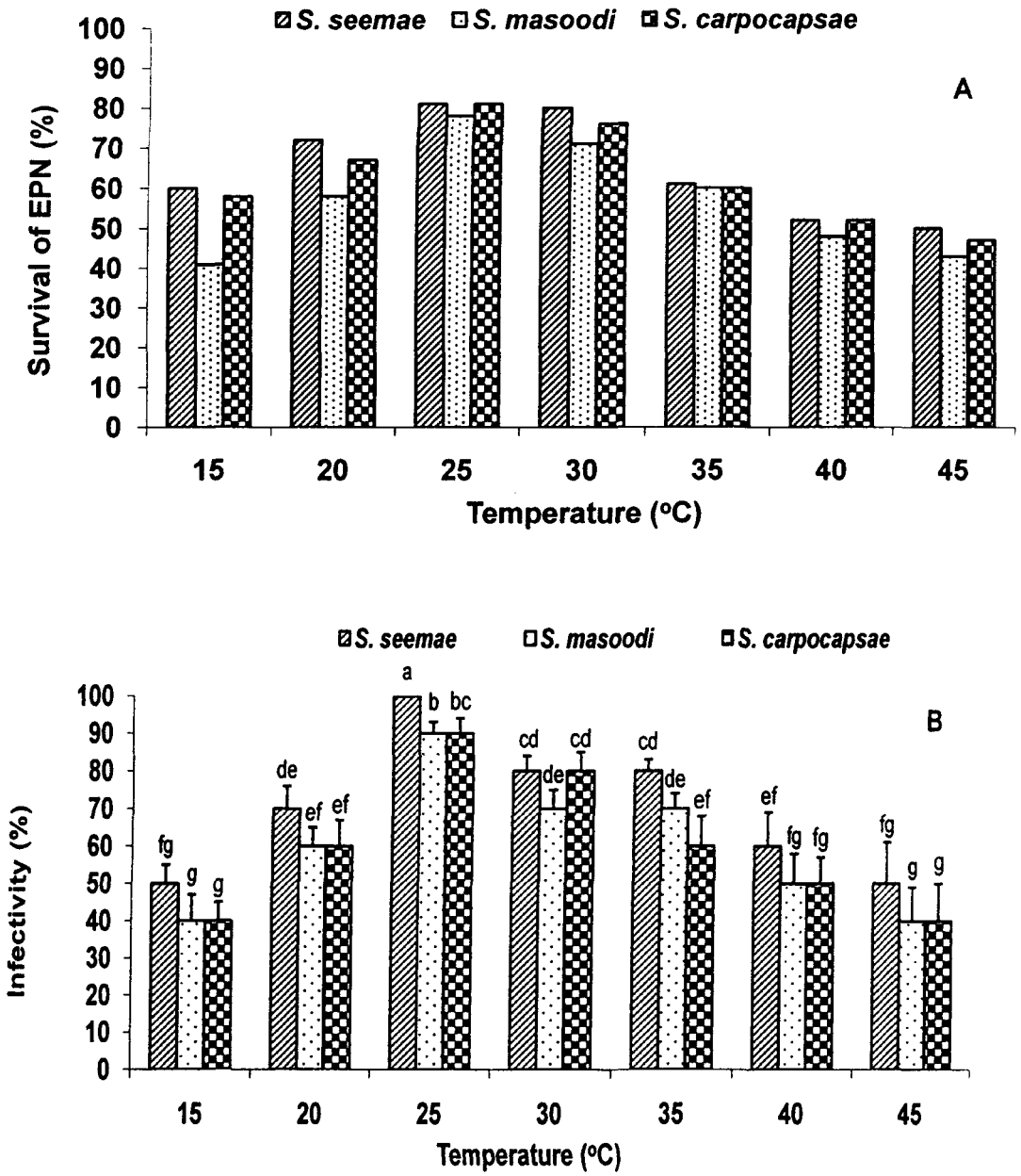



Figure 5.1. (A) Effect of different temperature on the survival of *Steinernema seemae*, *S. masoodi* and *S. carpocapsae* in soil. (B) Effect of different temperature on the infectivity of *Steinernema seemae*, *S. masoodi* and *S. carpocapsae* against *Helicoverpa armigera*. Control bars are not shown in the figure B whose values are zero. Different letters among means were considered significant at $P < 0.05$.

Exposure to extremes of temperature is damaging to nematodes, but the extent and nature of damage depends on the duration of exposure. All infective juveniles of *S. carpocapsae* exposed to 41°C for 1 h were killed (Schmiege 1963), while *S. carpocapsae* Arkansas isolate survived for two weeks in soil at 40°C (Gray & Johnson 1983). From the biological control perspective, the point at which irreversible heat or chill coma is induced in the nematodes is more important than the thermal death point. In general, *nematodes in deeper layers of soil will not be exposed to high lethal* temperatures (Kaya 1990); such temperatures are most likely to be encountered following foliar application, where they interact with the lethal effects of ultra violet radiation.

# 6 Survival of *Steinernema masoodi* and *S. carpocapsae* on pigeonpea and chickpea after foliar application


## ABSTRACT

The survival of *Steinernema masoodi* and *S. carpocapsae* was investigated after foliar application on pigeonpea and chickpea twigs, respectively, at flowering and fruiting stage. The concentration used was 2,500 infective juveniles (IJs)/ml of water. On pigeonpea, 19 and 3% survival of *S. masoodi* was after 30 and 60 minutes of spray in morning hours whereas 52 and 17% survival was recorded in evening hours, respectively. *S. masoodi* survival at 30 minutes post-spray in the morning was on par with 60 minutes of spray in the evening hours. Whereas on chickpea 40, 2 and 0% survival of *S. carpocapsae* were found at 1, 2 and 3 h post-spray in the morning hours whereas in the evening spray 70, 53 and 7% were found viable at 1, 2 and 3 h

post-spray, respectively. Addition of glycerine and UV retardant improved the survival of nematode to a little extent. However, results indicate that survival rate of IJs decreased fast and viability remained up to 3 h and in evening hours very few nematodes remained alive. Serious attempts are needed to improve the survival of nematodes after foliar spray by adding efficient adjuvant, humectant, antidesiccant and/or UV retardant for the management of aerial insect pests.

**6.1 Introduction**

Pigeonpea (*Cajanus cajan* (L.) Millsp.) and chickpea (*Cicer arietinum* L.) are important grain legumes in India and provide nutritious food, feed and fodder and constitute an integral component of subsistence farming system of the country (Asthana & Ali 1997). Among the various biotic constraints, the infestation and damage caused by insect pests is one of the major constraints towards their low production. About 20 insect pests have been reported to be of major importance at various growth stages of pigeonpea and chickpea inflicting heavy yield losses (Kumar & Nath 2003; Kooner et al. 2005). Average yield loss due to insect pests in chickpea has been estimated as 29% whereas in pigeonpea often exceed 50% (Kooner et al. 2005).

Inundative release of entomopathogenic nematodes (EPN) through flood irrigation was reported as successful method against *Helicoverpa zea* (Boddie) in Arkansas corn fields (Feaster & Steinkraus 1996). However, use of entomopathogenic nematodes to manage insect pests feeding on aerial parts of plant poses a considerable challenge as aboveground conditions are detrimental to nematodes (Arthurs et al. 2004). Infective juveniles (IJs) get inactivated quickly and are sensitive to extremes of physical environment,

particularly rapid desiccation (Womersley 1990), high temperature (Grewal et al. 1994), lethal UV radiation (Gaugler et al. 1992) and difficulty in establishing attraction gradients (Glazer 1992). Particularly, foliar application of entomopathogenic nematodes against aerial insect pests at 35-40°C needs to be resolved by improving their survival and efficacy. Efforts have been made to increase the survival of entomopathogenic nematodes through addition of adjuvant to minimize the above mentioned detrimental factors. In the present study an attempt was made to study the survival of *Steinernema masoodi* on pigeonpea and *S. carpocapsae* on chickpea after foliar spray at fruiting stage through including the use of an adjuvant, namely, UV retardant (fabric whitener) and glycerine at high temperature regimes.



## 6.2 Materials and methods

### *6.2.1 Nematodes and insect culture*

Greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), required for in vivo production of entomopathogenic nematodes, were reared on semi-synthetic diet as per procedure described by Ali et al. (2005b). *S. masoodi* and *S. carpocapsae* were multiplied on last instar larvae of *G. mellonella* and freshly harvested IJs was used in the present study.

### *6.2.2 S. masoodi survival on pigeonpea foliage*

Foliar application of *S. masoodi* was done on an early maturing variety of pigeonpea, UPAS 120, when the crop was at fruiting stage. About 8 ml of nematode suspension (concentration: 2500 IJs/ml) was sprayed on apical portion of each plant with hand compressed sprayer in an area of 20 $m^2$ at morning (7:00 am) and evening (4:30 pm) hours. Ten leaves and 2 pods per

plant were plucked after 0, 30 and 60 minutes of spray and dipped in 100 ml distilled water and left for one hour before counting the number of live IJs under binocular microscope. There were 18 treatments, viz., 3 formulations (EPN alone, EPN + Glycerine 1%, EPN + Glycerine 1% + 'Ujala supreme' 0.01% - a liquid fabric whitener (Jyothy Laboratories Ltd., Mumbai, Maharashtra, India) × 3 observation periods (0, 30 and 60 minutes) × 2 spray schedule (morning and evening hours) and replicated seven times. Temperature and prevailing RH (CIRAS-1 Portable Photosynthesis System, PP Systems, Herts, UK) was also recorded simultaneously.

*6.2.3 S. carpocapsae survival on chickpea foliage*

Foliar application of *S. carpocapsae* was applied on chickpea variety, SAKI 95–16, when the crop was at fruiting stage. Liquid EPN suspension (containing 2500 IJs/ml + glycerine 1% + Ujala 0.01%) was prepared in water spray. Sodium bicarbonate (0.5%) was also added to nullify the harmful effect of malic acid exudates from chickpea foliage. Spraying was done in an area of 3 m × 3 m plot in the morning (6:30 am) and evening (5:30 pm) hours. Two twigs along with pods per plant was cut with a scissor after 0, 1, 2 and 3 h post-spray and dipped in 100 ml distilled water and left for one hour in the morning spray and overnight in case of evening spray before final counts were made. There were 8 treatments: 4 observation periods (0, 1, 2 and 3 h) × 2 spray timing (morning and evening) and replicated five times. Records of temperature and prevailing RH was also taken simultaneously.

*6.2.4 Statistical analysis*

Data on survival of nematode were analysed using factorial ANOVA and means were separated using Tukey's LSD. Differences among means in experiments were considered significant at $P < 0.05$.

**6.3 Results**

*6.3.1 S. masoodi survival on pigeonpea foliage*

In morning sprays, *S. masoodi* IJs population reduced drastically from initial 236.1 to 44.4 within 30 minutes and only a few nematodes could survive after 60 minutes (18.8 and 2.9% survival, respectively, Table 6.1). Population of *S. masoodi* IJs was maximum (mean: 303.4) immediately after evening spray however significant reduction (mean: 158.4 and 51.6) was recorded after 30 and 60 minutes and resulted 52.2 and 17.0% survival from initial population, respectively. Nematode survival after 30 minutes in morning spray was on par with 60 minutes post-spray in the evening. Thus, spraying during evening hours was found superior with respect to nematode survival over the morning spray schedule. Addition of adjuvants were used to nullify the effect of external weather factors, like, desiccation and UV radiation to some extent. It was interesting to note that addition of glycerine has always resulted in lower EPN survival at any given exposure period. When UV retardant was also incorporated, it invariably gave rise to higher EPN survival than the other two treatments irrespective of time elapsed or period of spray. In morning spray, there were 258.6 IJs in this treatment as against 240.4 or 209.4 IJs. Similarly, spray during evening hours resulted in retaining 329.1 IJs on leaf surface as against 275.8 or 305.3 IJs in EPN alone or EPN + glycerine combination.

Table 6.1 Survival of *Steinernema masoodi* after spray on pigeonpea

| Spraying schedule (s) | Observation taken after spray (minutes) (t) | Temp. (°C) | Relative humidity (%) | EPN formulation (n)* | | | | | | Grand mean of live IJs (% survival |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | EPN alone | | EPN + Glycerine | | EPN + Glycerine + Ujala | | |
| | | | | Average no. of live IJs | % survival | Average no. of live IJs | % survival | Average no. of live IJs | % survival | |
| Morning | 0 | 25.0 | 75 | $240.4^{bcd}$ | 100 | $209.4^{cd}$ | 100 | $258.6^{abc}$ | 100 | 236.1 (100) |
| | 30 | 28.0 | 71 | $45.8^{g}$ | 19.1 | $31.7^{g}$ | 15.1 | $55.7^{g}$ | 21.5 | 44.4 (18.8) |
| | 60 | 30.5 | 65 | $16.1^{gh}$ | 6.7 | $0.3^{h}$ | 0.1 | $4.0^{h}$ | 1.5 | 6.8 (2.9) |
| Evening | 0 | 25.0 | 58 | $275.8^{abc}$ | 100 | $305.3^{ab}$ | 100 | $329.1^{a}$ | 100 | 303.4 (100) |
| | 30 | 21.5 | 60 | $148.8^{ef}$ | 53.9 | $142.8^{ef}$ | 46.8 | $183.6^{de}$ | 55.8 | 158.4 (52.2) |
| | 60 | 17.0 | 63 | $37.4^{g}$ | 13.6 | $68.1^{g}$ | 22.3 | $49.4^{gh}$ | 15.0 | 51.6 (17.0) |

* Different letters among means were considered significant at $P < 0.05$.

C.V. = 33.37%

| | CD ($p$ = 0.05) | SED |
|---|---|---|
| Spraying schedule (s) | 15.74 | 7.93 |
| Time elapsed post-spray (t) | 19.27 | 9.72 |
| Nematode formulation (n) | 19.27 | 9.72 |
| s × t | 27.26 | 13.74 |
| s × t × n | 47.22 | 23.81 |

*6.3.2 S. carpocapsae survival on chickpea foliage*

Survival of *S. carpocapsae* IJs when sprayed on chickpea was maximum (mean IJs count: 159.4 and 165.4) in morning and evening sprays and were on par (Table 6.2). Though with passing of time, the survival of nematode decreased but mortality rate was higher in morning than evening spray. Three

h post-spray populations of IJs was 11.4 in evening spray as compared to nil in morning spray, however, the population was on par with IJs population 2 h after morning spray.

Table 6.2 Survival of *Steinernema carpocapsae* after spray on chickpea

| Spraying schedule | Observation taken after spray (hours) | Temp. (°C) | Relative humidity (%) | Average no. of live IJs* | % Survival |
|---|---|---|---|---|---|
| Morning | 0 | 26.0 | 71 | 165.4[a] | 100 |
| | 1 | 27.5 | 69 | 65.8[c] | 39.8 |
| | 2 | 29.0 | 67 | 4.0[d] | 2.4 |
| | 3 | 32.0 | 64 | 0[d] | 0 |
| Evening | 0 | 24.0 | 78 | 159.4[a] | 100 |
| | 1 | 23.5 | 81 | 111.8[b] | 70.1 |
| | 2 | 22.0 | 85 | 83.8[c] | 52.6 |
| | 3 | 22.5 | 87 | 11.4[d] | 7.1 |
| | C.V. | | | 25.5% | |
| | SED | | | 11.5 | |
| | CD ($P = 0.05$) | | | 23.5 | |

* Different letters among means were considered significant at $P < 0.05$.

## 6.4 Discussion

In a pest management programme utilizing entomopathogenic nematodes as a component, some special considerations are needed. Ultraviolet radiation and dehydration are considered prime mortality factors resulting in 40-80% mortality or even more (Smits 1996) whereas relative humidity and temperature during and up to 8 h post-application were also predicted to influence rates of nematode infection obtained (Arthurs et al. 2004). In the present study, nematode survival was found lower in morning spray on pigeonpea foliage on which 2.9% population survived 1 h post-spray than

nematode survival ability at evening hours (17.0%). This may be attributed to the increase in solar radiation, temperature from 25.0 to 30.5°C and decrease in relative humidity from 75 to 65%. Reverse was the case with spray in the evening where higher EPN survival was recorded. Both the factors acted negatively on nematode survival. Among the two adjuvants, UV retardant seems to has performed better as more survival of EPN was observed irrespective of time elapsed between spray and observation.

On the basis of observation on pigeonpea, another trial was laid down on chickpea, where desiccation and inactivation of *S. carpocapsae* was found less so much so that 39.8 and 70.1% population survived after 1 h of spray while their survival prolonged for 2 h when sprayed during evening hours at 22°C. Gaugler et al. (1992) concluded that 60 minutes of exposure to direct sunlight inactivated *S. carpocapsae*. During morning spray, temperature gradually increased from 26.0 to 32.0°C within 3 h and humidity decreased from 71 to 64% (Table 6.2). Contrary to this, the corresponding figures for the evening spray were decreasing temperature from 24.0 to 22.5°C and increase in RH from 78 to 87% (Table 6.2). This has resulted in more survival of *S. carpocapsae* on chickpea leaf surface even after 3 h post-spray (0 and 11.4 IJs). Glazer (1991) reported that survival of IJs of *S. carpocapsae* All strain reduced to 20% after 4 h and to 0% after 8 h at 50-70% RH. Both the experiments on pigeonpea and chickpea suggest that the effects of sunlight, harmful UV rays or high temperature can be minimized by applying the nematodes at dusk as survival of both the species in the evening hours was better than morning spray schedule. However, maintaining high humidity (>

80% RH) and free water on the leaf surfaces is more difficult to achieve especially in dry farming ecosystem such as pigeonpea and chickpea.

Prabhuraj et al. (2005) recorded glycerol 0.1% as most appropriate antidesiccant resulting in 81.2% survival of *Heterorhabditis indica* after 2 h of foliar spray on chickpea foliage but survival reduced drastically after 4 h under field condition (12 to 26°C with 5-60% RH). In the present study, with passing of time, there were drastic reduction in *S. masoodi* population in all the treatments indicating that glycerine 1% and UV retardant 0.01% were not very effective in protecting the nematode survival beyond 3 h post-spray in morning but effective to some extent in evening. It suggests that some new molecules acting as adjuvant, humectant, antidesiccant and/or UV retardant has to be incorporated which, if used along with nematode, can prolong their survival on foliage.

# 7 Evaluation of *Steinernema masoodi* against soil-dwelling stage of *Helicoverpa armigera*


## ABSTRACT

The entomopathogenic nematode, *Steinernema masoodi* (Rhabditida: Steinernematidae), was evaluated for the suppression of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) at prepupal stage while undergoing pupation in soil. In laboratory, suppression (mean ± SE) of 71 ± 9.7% adult emergence was obtained with no significant differences at nematode concentrations 500, 1000, 2000, 3000 and 5000 infective juveniles (IJs)/60g soil/last instar larva. But at lower concentrations (0 to 500 IJs/60g soil/larva with increment of 50 IJs in treatments), varied percent suppression were recorded; highest (64 ± 7.4%) being at 450 IJs/larva and lowest (30 ± 3.3%) at 50 IJs/larva. By application of *S. masoodi* in chickpea microplots (40 cm × 40 cm), 70 ± 2.2% and 56 ± 3.5% suppression were obtained at

nematode concentrations 96,000 (≈ 6 × $10^9$ IJs/ha) and 64,000 (≈ 4 × $10^9$ IJs/ha) IJs/microplot, respectively which were at par. Whereas, *S. masoodi* at 32,000 (≈ 2 × $10^9$ IJs/ha) IJs/microlplot only 32 ± 7.0% suppression was achieved.

**7.1 Introduction**

*Helicoverpa armigera* (Hübner) is a polyphagous and devastating insect pest distributed over the tropics and subtropics of the world (Fitt 1989). In northern India, severe infestation of chickpea (*Cicer arietinum* L.) pod damage (up to 90%) by *H. armigera* has been reported (Sehgal & Ujagir 1990; Lal 1996). Entomopathogenic nematodes have been applied as foliar spray to control insect pests feeding on aboveground parts (Arthurs et al. 2004; Shapiro-Ilan et al. 2006). Poor to moderate levels of suppression were achieved when nematodes were applied to foliage to control *Helicovepra* (Bong & Sikorowski 1983; Richter & Fuxa 1990; Vyas et al. 2003). The survival of *Steinernema masoodi* on chickpea foliage remained up to few hours only (Ahmad et al. 2006a) thus limiting their effectiveness. The other possibility to control *H. armigera* is in the upper soil profile at a depth of 2.5 to 17.5 cm (Jayaraj 1982), the very own habitat of entomopathogenic nematodes, when final instar larva of *H. armigera* crawls to ground after completing their larval stages and enters crevices or loose soil for pupation in small earthen puparia. There is likelihood that nematode present in soil may encounter pupating larva and kill it prior to pupation. Cabanillas & Raulston (1996) suggested delivering nematodes through irrigation could be a potential system for suppressing *Helicoverpa* populations.

In laboratory studies, susceptibility of heliothinid prepupae and pupae to nematodes have been reported by several workers, for example, *H. armigera* and *H. zea* (Boddie) prepupae and/or pupae by *S. masoodi*, *S. glaseri*, *H. indica*, *S. riobrave* and *S. carpocapsae* (Kaya & Hara 1981; Cabanillas & Raulston 1994; Raulston et al. 2001; Ali et al. 2007; Banu et al. 2007). Testing under caged condition, Bell (1995) reported 66% reduction in *Heliothis virescens* adult emergence from soil under cotton plants treated with *S. riobrave*. Whereas in maize field, *S. riobrave* application to the soil resulted in 100 and 95% insect mortalities when 50% of *H. zea* larvae were late instars and when 10% of larvae had left ears to pupate, respectively (Cabanillas & Raulston 1995). Similarly, Feaster & Steinkraus (1996) achieved excellent results by applying *S. riobrave* to the soil in Arkansas corn field to control maize earworm (*H. zea*).

Recently, *S. masoodi* was isolated from sandy loam soil of pigeonpea field when temperature was soaring high (40–45°C) (Ali et al. 2005a). The thermal tolerance of this nematode suggests that it could play a potential role at high temperature regimes in the management of pupating *H. armigera* (Ali et al. 2007). Therefore, the aim of the present study was to test the effectiveness of different nematode concentrations of *S. masoodi* for the suppression of *H. armigera* at prepupal stage while undergoing pupation in soil under laboratory conditions and in microplots. This study could substantiate another novel approach to suppress *Helicoverpa* population in chickpea.

## 7.2 Materials and methods

### *7.2.1 Nematode and insect culture*

*Steinernema masoodi* was baited out from Kanpur, India (Ali et al. 2005a) and cultured on last instar larvae of *Galleria mellonella* L. as described by Kaya & Stock (1997), and freshly harvested infective juveniles (IJs) were used in the present study. The starter culture of *H. armigera* was collected from nearby pigeonpea and chickpea fields and reared on semi-synthetic diet as described by Armes et al. (1992). The last instar larvae of *H. armigera* were collected from chickpea fields and used in the Experiment 1; whereas laboratory-reared larvae were used in Experiment 2 and 3.

### *7.2.2 Laboratory experiments*

Experiment 1: The sterilized sandy loam soil weighing 60 g was put in plastic sample container (26 mm dia.) and sterilized distilled water was added to moist the soil (16.7% w/v moisture). The desired concentration of nematode (0, 500, 1000, 2000, 3000 or 5000 IJs/container) was poured over the soil surface and one last instar *H. armigera* larva was introduced in each container. Two twigs with 2 fruiting pods of chickpea were given as food to the larva and covered with perforated lid (Figure 7.1). The larva entering into the soil for pupation was noted down everyday up to one week; however the larva, which was killed before entering into the soil (Figure 7.2), was replaced with fresh ones because nematode-induced mortality on soil surface was obtained in some *H. armigera* larvae. The emergence of adult was checked everyday up to one month and percent suppression of adult emergence was calculated. Destructive observation of the soil was done on 30$^{th}$ day of larval

introduction. There were two replications in each treatment where one replicate comprised of a group of 10 last instar larvae of *H. armigera*.

Experiment 2: The set up was similar to Experiment 1 except (i) laboratory-reared larvae were used and (ii) ten nematode concentrations were used ranging from 0 to 500 IJs/container with the increment of 50 IJs. During the experimental period, mean room temperature was 30°C (range 28 to 33°C) and 58 ± 14.7% relative humidity (range 82 to 35%).

*7.2.3 Netted microplot field experiment*

Chickpea variety KWR 108 was sown on 16 December, 2006 at Main Farm, Indian Institute of Pulses Research (IIPR), Kanpur, India following recommended package and practices. At fruiting & podding stage of chickpea, 20 microplots (40 cm × 40 cm) were demarcated randomly and nylon nets were erected to safeguard the area under experimentation, protect the escape of introduced *H. armigera* larvae and trace the emerged adults (Figure 7.3). The treatment in the form of liquid suspension [32,000 (= 2 × $10^9$ IJs/ha), 64,000 (= 4 × $10^9$ IJs/ha) or 96,000 IJs/1600-$cm^2$ microplot (= 6 × $10^9$ IJs/ha)] was dispensed to the soil surface at evening time after 1800 hours IST on 24 March 2007 within the demarcated microplot, whereas control received one litre of water only. Then, 20 laboratory-reared last instar larvae of *H. armigera* were released on chickpea plants in each microplot. The aboveground larvae, which were killed before entering into the soil, were replaced with fresh ones. The adult emergence was checked daily up to one month. Each treatment had four replications where each replicate comprised of 20 last instar *H. armigera* larvae introduced in each microplot and entire set up was arranged in a complete randomized design.


Figure 7.1. Laboratory evaluation of *Steinernema masoodi* in the suppression of *Helicoverpa armigera* at the time of pupating in soil. (A) *S. masoodi* infective juveniles treated soil in plastic sample containers in trays containing final instar larvae of *H. armigera*. (B) Interior of sample container showing *H. armigera* fed on chickpea pods and faecal debris before pupating in *S. masoodi*-treated soil. (C) Adult *H. armigera* emerged from the control treatment.


Figure 7.2. Laboratory evaluation of *Steinernema masoodi* in the suppression of *Helicoverpa armigera* at the time of pupating in soil. (A) Final instar larva of *H. armigera* died while pupating into *S. masoodi*-treated soil. (B) Emerging infective juveniles over the body of *H. armigera* cadaver.

Figure 7.3. Nylon-netted chickpea microplots (40 cm × 40 cm) treated with *Steinernema masoodi* at the rate of 0, 2, 4 or 6 × $10^9$ IJs/ha in which 20 final instar larvae of *H. armigera* were released in each field cage. The adult emergence was monitored for one month and finally percent adult suppression was evaluated.

The meteorological data was pooled from the meteorological observatory installed at Main Farm, IIPR, Kanpur. The average climatic conditions (± SD) during the experimental period from 24 March to 24 April 2007 were as follows: ambient air temperature 30 ± 8.3°C (range 16 to 42°C); relative humidity 43 ± 13.7% (range 19 to 71%); evaporation rate 8.8 ± 1.8 mm/day (range 4.4 to 12 mm/day); sunshine 9.5 ± 1.0 h (range 6.5 to 11 h) and record of no rainfall except one day (0.2 mm).

*7.2.4 Statistical analysis*

The data presented as percent mortality was first corrected following Abbott's formula (Abbott 1925), normalized using arcsine transformation, and then subjected to general linear model (GLM) univariate analysis of variance (SPSS 2002). Difference between means of each experiment was separated by Tukey's HSD test and considered significant at an alpha level of 0.05.

**7.3 Results**

*7.3.1 Laboratory experiments*

In Experiment 1 at all nematode concentrations (mean ± SE), 71 ± 9.7% suppression of adult emergence was achieved (Figures 7.4 & 7.5). Statistically, percent suppression of adult emergence was not significantly different among nematode concentrations ranging 500 to 5000 IJs ($F$ = 29.77; df = 5, 6; $P$ < 0.001) compared to untreated control (Table 7.1). However, narrowing down the range of nematode concentrations as explored in Experiment 2, variable percent suppression of *H. armigera* adult emergence were obtained ($F$ = 11.37; df = 10, 11; $P$ < 0.001). Overall, 48 ± 4.0% suppression of adult emergence was recorded at all nematode concentrations. The highest suppression of 64 ± 7.4% was achieved at 450

IJs/container but lowest (30 ± 3.3%) was recorded at 50 IJs/container whereas suppression ranged 45 to 63% at nematode concentrations 100 to 500 IJs/container (Table 1).

**Table 7.1** Per cent suppression of *Helicoverpa armigera* adult emergence by *Steinernema masoodi* at prepupal stage in soil under laboratory conditions

| Nematode concentration (IJs/60g soil/container) | Percent suppression of adult emergence* (SE) |
|---|---|
| Experiment 1 | |
| 0 | 0.8 (0) b |
| 500 | 80.4 (8.8) a |
| 1000 | 76.3 (12.9) a |
| 2000 | 89.2 (0) a |
| 3000 | 89.2 (0) a |
| 5000 | 89.2 (0) a |
| Experiment 2 | |
| 0 | 0.6 (0) c |
| 50 | 29.9 (3.3) bc |
| 100 | 45.0 (11.8) ab |
| 150 | 45.0 (5.8) ab |
| 200 | 56.8 (0) ab |
| 250 | 53.8 (3.0) ab |
| 300 | 63.4 (0) a |
| 350 | 57.1 (6.3) ab |
| 400 | 50.9 (5.9) ab |
| 450 | 64.2 (7.4) a |
| 500 | 60.1 (3.3) ab |

* Within a column, means (Abbott corrected and arcsine transformed values) followed by the same lowercase letter are not significantly different, ANOVA, Tukey's HSD test at $P < 0.05$. Experiments 1 and 2 were analyzed separately. Means of two replicates where each replicate comprised of 10 last instar larvae of *H. armigera*.

Figure 7.4. Mortality of soil-dwelling stages (last instar larva or prepupa) of *Helicoverpa armigera* while pupating in soil caused by infective juveniles of *Steinernema masoodi* and multiplying nematode progeny upon *H. armigera* pupa.

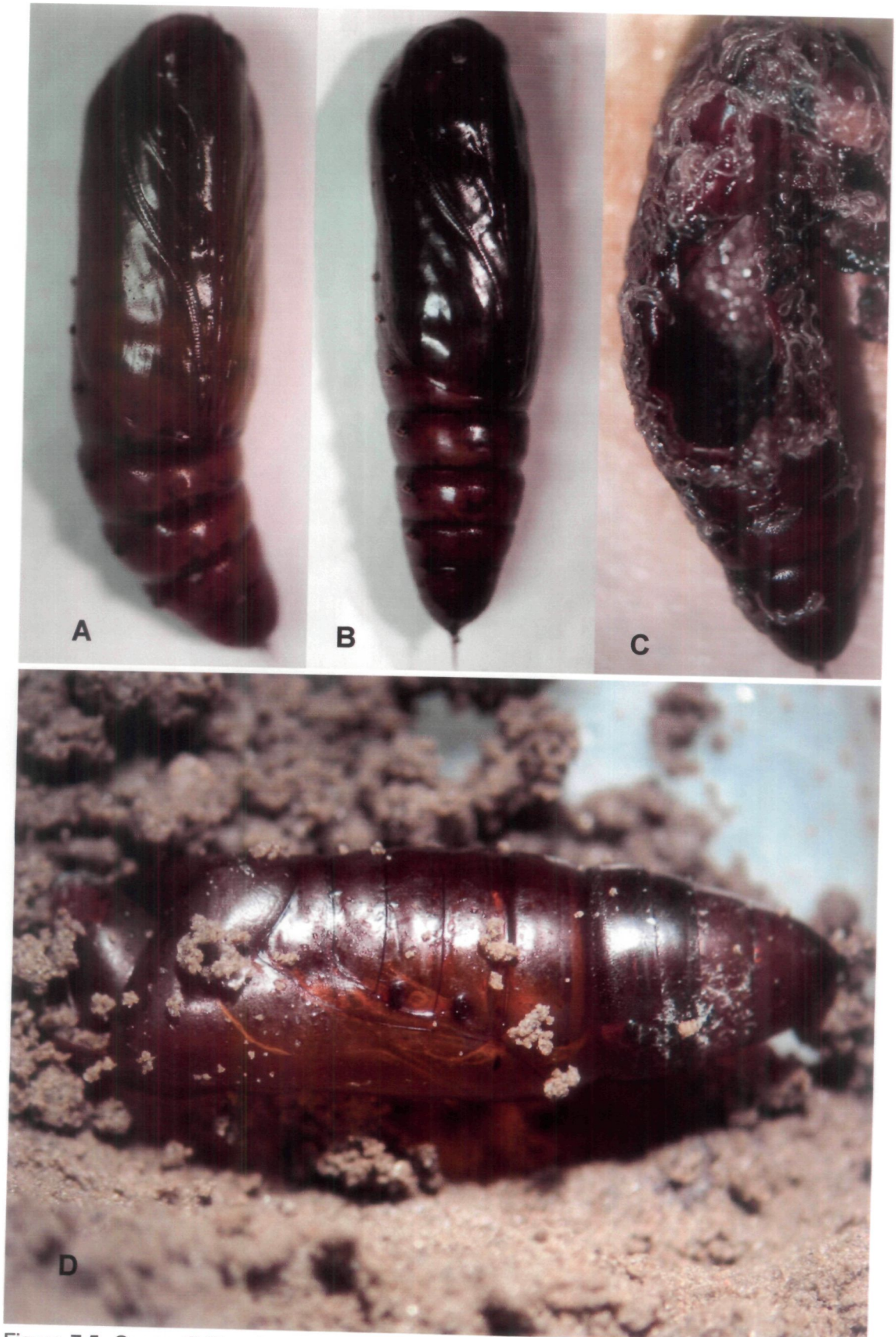



Figure 7.5. Susceptibility of pupal stage of *Helicoverpa armigera* by *Steinernema masoodi*. (A) Healthy pupa. (B) Darkened colouration of pupa after *S. masoodi* infection. (C) Multiplication of *S. masoodi* over the pupal case. (D) Empty pupal case left over by *S. masoodi* progeny after multiplication.

*7.3.2 Netted microplot field experiment*

The field experiment in microplot resulted in 40 ± 7.0% suppression of adult emergence pooled across all nematode concentrations; In the field plot studies, larval mortality was significantly affected by treatment with *S. masoodi* ($F$ = 56.26; df = 3, 12; $P < 0.001$). Suppression of adult emergence 70 ± 2.2 and 56 ± 3.5% was obtained at nematode concentration 96,000 (=6 × $10^5$ IJs/$m^2$) and 64,000 IJs/microplot (=4 × $10^5$ IJs/$m^2$), respectively which was on par with each other. Whereas, at nematode concentration 32,000 IJs/microlplot (=2 × $10^5$ IJs/$m^2$), suppression of 32 ± 7.0% was recorded (Figure 7.6).

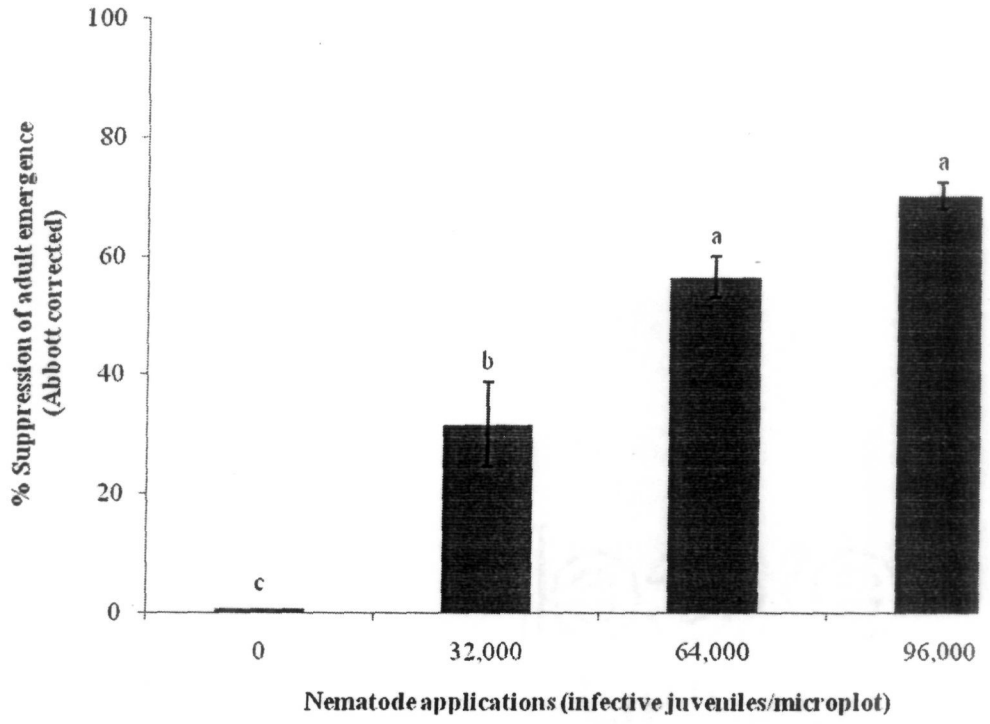



Figure 7.6. Percent suppression of *Helicoverpa armigera* adult emergence by *Steinernema masoodi* at prepupal stage in chickpea microplot (40 cm × 40 cm). Infective juveniles were applied to soil surface and *H. armigera* last instar larvae were released in netted microplot, and adult emergence was monitored up to one month. Bars (means, Abbott corrected and arcsine transformed values, of four replicates where each replicate comprised of 20 *H. armigera* larvae) indicated with the same letter are not significantly different according to Tukey's HSD test at $P < 0.05$. Thin bars are standard errors of means.

## 7.4 Discussion

The results obtained through these experiments were highly enlightening and could play a critical role in the management of *H. armigera* at the time when larva is finding a hiding place in crack, crevices or loose soil in the field to undergo pupation.

In Experiment 1, out of the broad nematode treatments applied starting from 500 to 5000, even lowest nematode concentration of 500 IJs was able to prevent the adult emergence up to 80 ± 8.8%. Therefore Experiment 2 was carried out to find the efficacy level at lower range of dosages, in which variable suppression (45 to 64%) was obtained at nematode concentrations 100 to 500 IJs/container. In present study, percent suppression is less than those reported by Cabanillas & Raulston (1994) and Raulston et al. (2001). They got 100% mortality of *H. zea* in petri dish bioassay by exposure to 100 IJs of *S. riobrave*/prepupa. In our case, less suppression could be due to larval mortality obtained in control treatment resulting in overall lowering percent suppression (vs. no mortality in control (Cabanillas & Raulston 1994; Raulston et al. 2001)), and use of different nematode species (*S. masoodi* vs. *S. riobrave*) suited to local insect pest (*H. armigera* vs. *H. zea*).

In microplot field experiment, adult emergence was not suppressed much (70 ± 2.2%) as expected even at higher dosage ($6 \times 10^5$ IJs/$m^2$). This may due to the harsh climatic condition (high aboveground temperature, low %RH, high rate of water evaporation, no rainfall and dry sandy loam soil) prevailing during the period of experimentation. As no irrigation was given after the nematode treatment, restriction in movement of nematode due to very low soil moisture resulted in less suppression of adult emergence. However, our

results corroborates with Feaster & Steinkraus (1996) who reported 78.5% *H. zea* mortality at the application of 5.2 × $10^5$ IJs of *S. riobrave*/$m^2$ in sweet corn. Whereas, Cabanillas & Raulston (1996) obtained higher insect mortalities by application of *S. riobrave* at a concentration of 2 × $10^5$ IJs/$m^2$ via in-furrow irrigation (95%) than when it was applied after irrigation (84%) or before irrigation (56%). Cabanillas & Raulston (1995) reported timing soil applications of nematode with the life cycle of the target insect is a key efficacy factor.

*Helicoverpa armigera* larvae after completing its feeding on aerial parts like leaves, pods, squares, bolls, etc., also drop down to soil and pupae in earthen cocoons. There is every likelihood that pupating larva will come into contact with nematodes and die, if nematodes are present in the soil. Thus nematodes will check adult emergence and further multiplication of the pest. Th innundative release of nematode may have application for commercial crop protection, but the larvae must complete their development before being controlled and drop down to soil, thereby reducing insect pest pressure for the next cropping cycle – an approach that is feasible only in a long-term integrated pest management strategy for a wider area and diverse cropping system where *H. armigera* is the pest for most of the crops grown.

In semi-arid tropics, chickpea is grown under rainfed cool-weather crop or dry climate crop and on residual moisture (Ahlawat et al. 2003; Smithson et al. 1985) but no irrigation is needed to raise the crop. If desired, only one time irrigation is given during flowering and/or fruiting period, which is the most appropriate time of nematode application as high incidence of *H. armigera* is expected during this period. To test this hypothesis, more field experiments

are needed to be addressed in greater detail before the implementation of inundative release of this biocontrol agent for the suppression of *H. armigera* in chickpea cropping system.

# 8 In vivo production of *Steinernema carpocapsae* and *Heterorhabditis indica* in *Corcyra cephalonica* and *Galleria mellonella* and economics of nematodes production


## ABSTRACT

In vivo production of *Steinernema carpocapsae* and *Heterorhabditis indica* in *Corcyra cephalonica* (rice meal moth) and *Galleria mellonella* (greater wax moth), respectively were carried out under laboratory conditions. Effect of overcrowding of nematode-infected cadavers kept at modified White trap were also undertaken. A maximum of 31,907 IJs/larva was obtained by the placement of 1.5 g *C. cephalonica* cadavers on petriplate ($n$ = 37). The average production of 23,926 IJs/larva was obtained when 2 g cadavers were kept in petriplate ($n$ = 5) followed by 1 g (14,738 IJs/larva), 3 g (7,533 IJs/larva) and 6 g (4,845 IJs/larva). Highly significant results were obtained

regarding production of *H. indica* with respect to larval weight of *G. mellonella*. A total of 6131 × $10^5$ IJs were harvested from 3,834 *G. mellonella* larvae (mean ± SE: 1.6 ± 0.13 × $10^5$ IJs/larva) with an average production of 7.0 ± 0.51 × $10^5$ IJs/g of cadaver.

Economics of nematode production was worked out based on the progeny obtained in the present study. A total of 104,489 *C. cephalonica* larvae would be required to produce standard nematode dose of 2.5 × $10^9$ IJs/ha – the cost of production of which comes to about Rs. 1950 (equivalent to US$ 39.1) excluding labour charges. Whereas, 15,244 *G. mellonella* larvae (cost of production Rs. 2363 ≈ US$ 47.3) will be required for one hectare treatment. Therefore, in case of greater nematode demand, cottage industries could be set up wherein more infrastructure will be needed to run the nematode production technology based on *Corcyra* or *Galleria* as insect host. Further, their widespread installation will open avenues for rural employment generation at grass root level.

**8.1 Introduction**

Entomopathogenic nematodes are potent biopesticides that can be mass-produced using in vivo (culture in live insect hosts) or in vitro (solid or liquid) culture methods (Friedman 1990; Ehlers 2001; Shapiro-Ilan & Gaugler 2002; Gaugler & Han 2002). Although in vitro production has the advantage of economy of scale (Friedman 1990), in vivo culture is still essential to numerous scientific and industrial interests. Relative to the other methods, in vivo production requires less capital, technical expertise and are suitable for laboratory-scale production and among many small nematode-producing companies. Therefore, it is also arguably the most appropriate technology for

grower cooperatives and for developing countries where labour is less expensive (Gaugler et al. 2000; Gaugler & Han 2002).

The production technology has been described by a number of authors (Dutky et al. 1964; Poinar 1979; Woodring & Kaya 1988; Lindegren et al. 1993; Flanders et al. 1996), all of which use White trap (White 1927) as a basis for the method. In vivo production is a two-dimensional process, which translates into a system of shelves and trays (Friedman 1990). In general, insects are inoculated on a tray or dish lined with an absorbent substrate, and after 2 to 7 days infected cadavers are moved to a harvest dish (White trap).

The most common and most studied insect host used for entomopathogenic nematode culture is greater wax moth, *Galleria mellonella* (L.), because of its high susceptibility to most nematodes, wide availability, ease in rearing, and high yields of IJ stages (Flanders et al. 1996; Shapiro-Ilan & Gaugler 2002). Other than *G. mellonella*, mealworm (*Tenebrio molitor* L.) (Shapiro-Ilan & Gaugler 2002) and rice meal moth, *Corcyra cephalonica* (L.) (Ali et al. 2008) are also used as insect hosts.

Factors that can affect production include method of inoculation, nematode concentration, and host density. Inoculation can be accomplished by applying nematodes (e.g., by pipet) to an absorbent substrate on which insects are added, by immersing the hosts in a nematode suspension or, in some cases, by applying the nematodes to the insect's food (Shapiro-Ilan & Gaugler 2002). The concentration of nematodes exposed to hosts during inoculation clearly has been shown to be positively related to the resulting number of infected insects (Flanders et al. 1996; Shapiro et al. 1999). However, it has also been suggested that too high concentration results in increased contamination and

decreased nematode infection or yield (Woodring & Kaya 1988). Furthermore, reports on the effects of nematode concentration on yield have varied (Zervos et al. 1991; Flanders et al. 1996; Boff et al. 2000). Flanders et al. (1996) did not observe effects of host density on nematode yield. Yet, due to nematode's requirement for oxygen (Burman & Pye 1980), one would expect that overcrowding of hosts could lead to reduced production. Shapiro-Ilan et al. (2002a) hypothesized maximized yield per host at intermediate host densities (at greatest density that does not have overriding crowding effects).

Our objective was to optimize production of local isolates of *S. carpocapsae* and *H. indica* in *C. cephalonica* and *G. mellonella*, respectively by determining effects of host density on final yield and to analyze the economics of their production. Focus was laid on *S. carpocapsae* and *H. indica* because they were indigenous in origin and locally available. Experiments were conducted at a scale, which deemed relevant for generating field trial material.

## 8.2 Materials and methods

### *8.2.1 Nematodes and insects culture*

*Steinernema carpocapsae* Kanpur isolate and *H. indica* Meerut isolate were cultured on last instar larvae of *G. mellonella* as per procedure of Kaya & Stock (1997). Emerged infective juveniles (IJs) were surface sterilized in 0.1% Hyamine® solution and stored in distilled water in tissue culture flasks. Up to one-week-old cultures were used in the experiments.

*Corcyra cephalonica* and *G. mellonella*, required for in vivo production of these nematodes, were reared on semi-synthetic diets as per procedure described by Ali et al. (2005b) and Prasad et al. (2008), respectively.

*8.2.2 S. carpocapsae production in C. cephalonica*

Six experiments were carried out to estimate the optimum production of *S. carpocapsae* in *C. cephalonica* larvae (Table 8.1).

Table 8.1 Detail of experiments on in vivo production of *S. carpocapsae* in *C. cephalonica* larvae

| Expt. No. | No. of larvae placed on White trap, larval weight | Inoculation (IJs/petriplate) | Replicates | Total larvae used |
|---|---|---|---|---|
| 1 | No. constant [50], wt. variable [1 pp*] | 500 | 22 | 1100 |
| 2 | No. variable, wt. constant [1.5g × 1 pp] | 500 | 37 | 1620 |
| 3 | No. variable, wt. constant [6g = 1g × 6 pp] | 1000 | 5 | 831 |
| 4 | No. variable, wt. constant [6g = 2g × 3 pp] | 1000 | 5 | 938 |
| 5 | No. variable, wt. constant [6g = 3g × 2 pp] | 1000 | 5 | 976 |
| 6 | No. variable, wt. constant [6g = 6g × 1 pp] | 1000 | 5 | 814 |

* No. of Petriplates (pp) used to keep cadavers on modified White trap in one replicate

*Steinernema carpocapsae* was mass produced on last instar larvae of *C. cephalonica*. One ml of nematode suspension containing approx. 500 IJs/ml (or 1000 IJs/ml in case of experiments # 3 to 6) was evenly distributed on a 9-cm filter paper in bottom lid of Petriplate (dia. 100 mm × 15-mm height). In experiments # 1 and 2, 25 larvae of *C. cephalonica* were added to each Petriplate whereas 50 larvae were introduced in experiments # 3 to 6 and covered with lid. Petriplates were kept in BOD at 28 ± 1 °C and 92% RH. After 2 days of introduction, dead larvae were removed and placed on modified White trap. Emerging IJs were harvested every alternate day up to 2 weeks, rinsed in a beaker and quantified by serial dilution in counting dish under binocular microscope and mean values ($n$ = 5) were calculated.

*8.2.3 H. indica production in G. mellonella*

*Heterorhabditis indica* was cultured on final instar larvae of *G. mellonella*. Five ml nematode suspension containing approx. 1,000 IJs/ml was evenly distributed on filter paper (dia. 195 mm) in a Petriplate (dia. 200 × 15-mm height) and 100 larvae of *G. mellonella* were added to it, secured with cover lid and kept in BOD at 28 ± 1 °C and 92% RH. *H. indica*-infected dead larvae were transferred 2-days later to plastic trays (254 × 360 mm) and incubated for 5 days and then placed on moist netted plastic trays to harvest emerging nematodes (Figure 8.1). Nematode quantification was done as stated earlier.

*8.2.4 Statistical analysis*

Data on mass production of nematodes were analysed by ANOVA and means were separated using LSD. Differences among means in experiments were considered significant at $P < 0.05$.

## 8.3 Results and discussion

*8.3.1 S. carpocapsae production in C. cephalonica*

In experiment # 1 when the number of infected larvae kept constant with variation in larval weight (mean wt. of 50 larvae: 1.24 ± 0.045 g), an average yield (mean ± SE) of 8.03 ± 0.81× $10^5$ IJs of *S. carpocapsae* was obtained from *C. cephalonica* larvae with the average production of 6.54 ± 0.65 × $10^5$ IJs/g (Table 8.2). On an average, 16,057 IJs/larva was produced from insect larvae. Whereas in experiment # 2 at constant weight of infected larvae (mean number of 1.5 ± 0.00061 g larvae: 44; range 57 to 32 larvae) with variable number used, average yield of 14.04 ± 0.33 × $10^5$ IJs were obtained with the average production of 9.34 ± 0.22 × $10^5$ IJs per g (Table 8.3). On an average, 31,907 IJs/larva was produced.

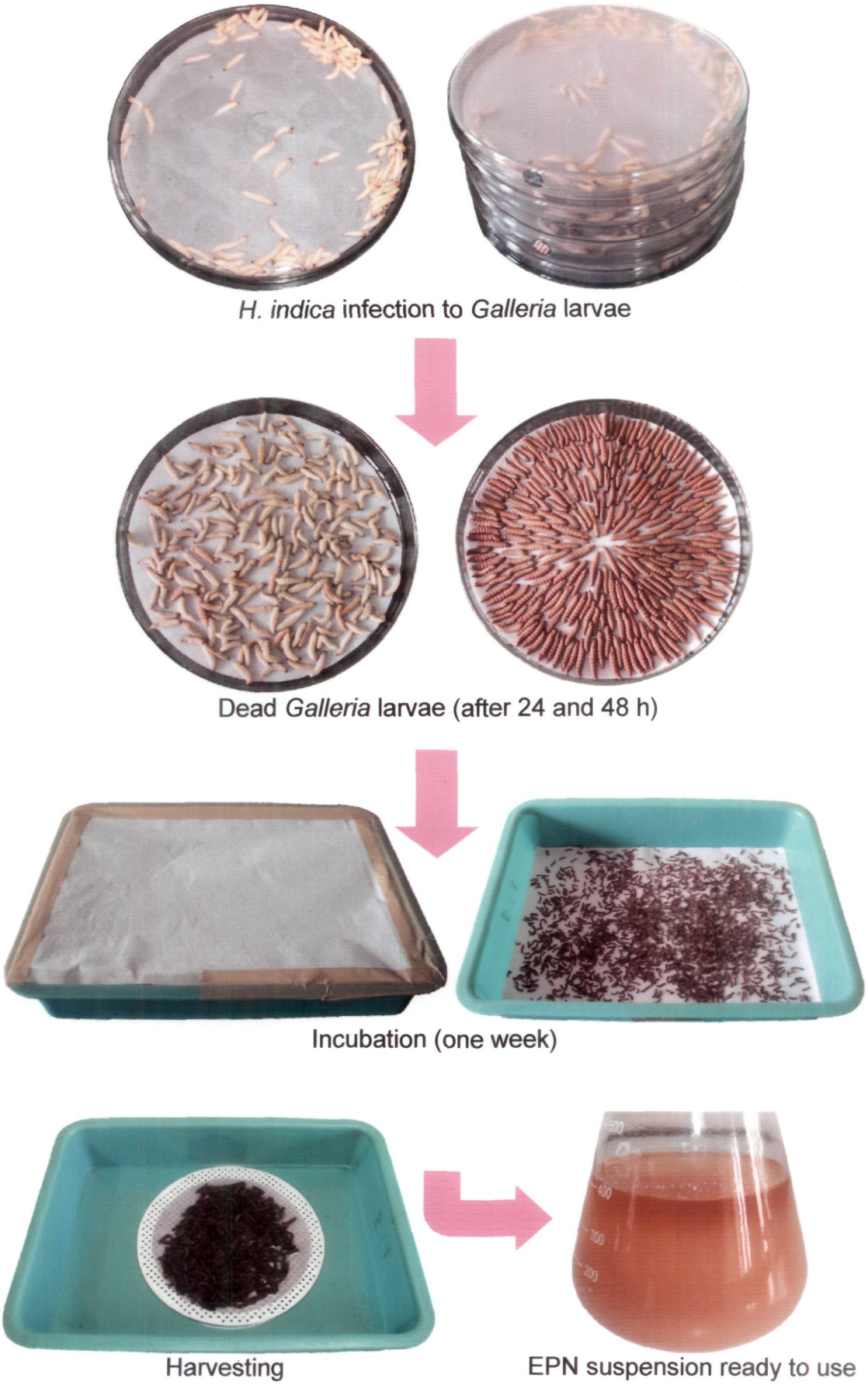



Figure 8.2 In vivo production of *Heterorhabditis indica* in final instar larvae of *Galleria mellonella*.

Highly significant results were obtained in the experiments # 3 to 6 where predefined cadavers weight (1, 2, or 3 g) placed in modified White trap had profound effect on *S. carpocapsae* production (Table 8.4). Highest nematode ($39.0 \pm 2.63 \times 10^5$ IJs) was harvested from traps having 2 g of cadavers followed by $27.7 \pm 4.56 \times 10^5$ nematodes from 1 g whereas placement of cadaver weight of 3 and 6 g resulted in poor production ($14.7 \pm 1.51 \times 10^5$ and $8.3 \pm 0.76 \times 10^5$ IJs). Overall, average production of 23,926 IJs was obtained from a single *Corcyra* larva kept in aggregate of 2 g of cadavers weight followed by 1 g (14,738 IJs/larva), 3 g (7,533 IJs/larva) and 6 g (4,845 IJs/larva).

*8.3.2 H. indica production in G. mellonella*

Highly significant yield of *H. indica* from *G. mellonella* was obtained with respect to larval weight. A total yield of $6131.0 \times 10^5$ IJs were harvested from 3,834 *G. mellonella* larvae with the average production of $1.6 \pm 0.16 \times 10^5$ IJs/larva and $7.0 \pm 0.5 \times 10^5$ IJs/g (Table 8.5).

Table 8.2 Production of *Steinernema carpocapsae* from *Corcyra* larvae (Experiment # 1)

| Replication number | Weight of 50 *Corcyra* larvae (g) | Total yield (IJs) | Yield/g |
|---|---|---|---|
| 1 | 1.15 | 552000 | 480000.0 |
| 2 | 1.34 | 515400 | 384626.9 |
| 3 | 1.38 | 416120 | 301536.2 |
| 4 | 1.09 | 270750 | 248394.5 |
| 5 | 1.13 | 412190 | 364769.9 |
| 6 | 1.71 | 865500 | 506140.4 |
| 7 | 1.10 | 271900 | 247181.8 |
| 8 | 1.21 | 296460 | 245008.3 |
| 9 | 1.01 | 390840 | 386970.3 |
| 10 | 1.30 | 869705 | 669003.6 |
| 11 | 1.41 | 880988 | 624814.5 |
| 12 | 1.51 | 743114 | 492128.7 |
| 13 | 1.39 | 1481916 | 1066126.6 |
| 14 | 1.40 | 1336120 | 954371.4 |
| 15 | 0.79 | 694780 | 879468.4 |
| 16 | 1.02 | 997180 | 977627.5 |
| 17 | 1.06 | 1075780 | 1014886.8 |
| 18 | 1.08 | 1010680 | 935814.8 |
| 19 | 1.02 | 994420 | 974921.6 |
| 20 | 1.41 | 1087240 | 771092.2 |
| 21 | 1.38 | 954840 | 691913.0 |
| 22 | 1.31 | 1545180 | 1179526.7 |
| **Average** | **1.24** | **802,868** | **654,378.4** |
| SE | 0.045 | 81,402 | 64,935.4 |

Table 8.3 Production of *Steinernema carpocapsae* from *Corcyra* larvae (Experiment # 2)

| Replication number | Weight of *Corcyra* larvae (g) | No. of *Corcyra* larvae | Total yield (IJs) | Yield/g |
|---|---|---|---|---|
| 1 | 1.5077 | 51 | 1313670 | 871307.3 |
| 2 | 1.5007 | 52 | 1178180 | 785087.0 |
| 3 | 1.5018 | 54 | 1335710 | 889406.0 |
| 4 | 1.5032 | 52 | 1066930 | 709772.5 |
| 5 | 1.5035 | 55 | 1328200 | 883405.4 |
| 6 | 1.5006 | 55 | 1232650 | 821438.1 |
| 7 | 1.5051 | 52 | 1414750 | 939970.8 |
| 8 | 1.5054 | 57 | 1728900 | 1148465.5 |
| 9 | 1.4995 | 49 | 1713800 | 1142914.3 |
| 10 | 1.5001 | 46 | 1472160 | 981374.6 |
| 11 | 1.5002 | 34 | 1303520 | 868897.5 |
| 12 | 1.5009 | 41 | 1393400 | 928376.3 |
| 13 | 1.5092 | 40 | 1376400 | 912006.4 |
| 14 | 1.5006 | 38 | 1383500 | 921964.5 |
| 15 | 1.5047 | 42 | 1169680 | 777351.0 |
| 16 | 1.5032 | 42 | 1078880 | 717722.2 |
| 17 | 1.5038 | 41 | 1252800 | 833089.5 |
| 18 | 1.5036 | 42 | 962800 | 640329.9 |
| 19 | 1.4992 | 43 | 1382067 | 921869.4 |
| 20 | 1.4993 | 41 | 1427500 | 952111.0 |
| 21 | 1.498 | 47 | 1443333 | 963506.9 |
| 22 | 1.4987 | 48 | 1714133 | 1143746.8 |
| 23 | 1.4969 | 41 | 1280200 | 855234.2 |
| 24 | 1.5092 | 41 | 1693133 | 1121874.7 |
| 25 | 1.5091 | 40 | 1759233 | 1165750.0 |
| 26 | 1.5032 | 42 | 1374400 | 914316.1 |
| 27 | 1.5087 | 46 | 1558767 | 1033185.3 |
| 28 | 1.5011 | 44 | 1403633 | 935069.8 |
| 29 | 1.5091 | 36 | 1336267 | 885472.6 |
| 30 | 1.5048 | 47 | 1676000 | 1113769.3 |
| 31 | 1.4987 | 38 | 1680267 | 1121149.4 |
| 32 | 1.5017 | 34 | 1280533 | 852722.5 |
| 33 | 1.5021 | 32 | 1394367 | 928278.2 |
| 34 | 1.5081 | 35 | 1576833 | 1045576.1 |
| 35 | 1.5065 | 36 | 1165733 | 773802.4 |
| 36 | 1.5075 | 48 | 1437867 | 953808.7 |
| 37 | 1.5098 | 38 | 1654040 | 1095535.8 |
| **Average** | **1.5034** | **44** | **14,03,898** | **933774.5** |
| SE | 0.00061 | 1.09 | 33306 | 22088.2 |

Table 8.4 Effect of weight of cadavers on production of infective juveniles from *Corcyra* larvae kept at modified trap (Experiment # 3, 4, 5 and 6)

| Expt. # | *n* | Weight of *Corcyra* larvae (g) | No. of *Corcyra* larvae | Total yield (IJs) | Yield/g |
|---|---|---|---|---|---|
| 3 | 1 | 6.0 = 1g × 6 | 158 | 1448000 | 241333.2 |
| | 2 | 6.0 = 1g × 6 | 141 | 2064000 | 344000.0 |
| | 3 | 6.0 = 1g × 6 | 186 | 3614668 | 602444.4 |
| | 4 | 6.0 = 1g × 6 | 222 | 2869332 | 478222.4 |
| | 5 | 6.0 = 1g × 6 | 231 | 3857332 | 642888.8 |
| | | Average | **188** | **2770668** | **461777.6** |
| | | SE | | **4.56** | |
| 4 | 1 | 6.0 = 2g × 3 | 137 | 3060000 | 510000.0 |
| | 2 | 6.0 = 2g × 3 | 130 | 3610000 | 601666.7 |
| | 3 | 6.0 = 2g × 3 | 127 | 4420000 | 736666.7 |
| | 4 | 6.0 = 2g × 3 | 180 | 3940000 | 656666.7 |
| | 5 | 6.0 = 2g × 3 | 240 | 4470000 | 745000.0 |
| | | Average | **163** | **3900000** | **650000.0** |
| | | SE | | **2.63** | |
| 5 | 1 | 6.0 = 3g × 2 | 180 | 1104000 | 184000.0 |
| | 2 | 6.0 = 3g × 2 | 178 | 1297600 | 216266.6 |
| | 3 | 6.0 = 3g × 2 | 173 | 1631200 | 271866.6 |
| | 4 | 6.0 = 3g × 2 | 227 | 1971200 | 328533.4 |
| | 5 | 6.0 = 3g × 2 | 218 | 1340800 | 223466.6 |
| | | Average | **195** | **1468960** | **244826.6** |
| | | SE | | **1.51** | |
| 6 | 1 | 6.0 = 6g × 1 | 174 | 846667 | 141111.1 |
| | 2 | 6.0 = 6g × 1 | 133 | 1048333 | 174722.2 |
| | 3 | 6.0 = 6g × 1 | 205 | 820900 | 136816.7 |
| | 4 | 6.0 = 6g × 1 | 208 | 858000 | 143000.0 |
| | 5 | 6.0 = 6g × 1 | 136 | 568333 | 94722.2 |
| | | Average | **171** | **828447** | **138074.4** |
| | | SE | | **0.76** | |

Table 8.5 In vivo production of *Heterorhabditis indica* from last instar larvae of *Galleria mellonella*

| Repl. # | No. of *G. mellonella* larvae | Larval weight (g) | Total yield ($\times 10^5$ IJs) | Yield/g ($\times 10^5$ IJs) | Yield/larva ($\times 10^5$ IJs) |
|---|---|---|---|---|---|
| 1 | 297 | 86.520 | 402.74 | 4.65 | 1.36 |
| 2 | 232 | 50.470 | 561.12 | 11.12 | 2.42 |
| 3 | 308 | 68.257 | 558.00 | 8.17 | 1.81 |
| 4 | 390 | 83.585 | 436.00 | 5.22 | 1.12 |
| 5 | 263 | 59.031 | 442.18 | 7.49 | 1.68 |
| 6 | 375 | 94.637 | 668.80 | 7.07 | 1.78 |
| 7 | 499 | 115.687 | 762.50 | 6.59 | 1.53 |
| 8 | 75 | 18.205 | 174.72 | 9.60 | 2.33 |
| 9 | 200 | 40.952 | 217.80 | 5.32 | 1.09 |
| 10 | 200 | 48.676 | 344.44 | 7.08 | 1.72 |
| 11 | 200 | 51.125 | 458.50 | 8.97 | 2.29 |
| 12 | 200 | 49.136 | 405.59 | 8.25 | 2.09 |
| 13 | 200 | 48.014 | 408.64 | 8.51 | 2.04 |
| 14 | 395 | 61.201 | 289.93 | 4.74 | 0.73 |
| **Total** | | **875.496** | **6130.96** | **7.00** | **1.59** |
| | **SE** | | | **0.51** | **0.13** |

*8.3.3 Economics of nematode production*

*Corcyra cephalonica* are reared in wooden rectangular box containing 2.5 kg coarse jowar (*Sorghum vulgare* Pers.) grains from which approx. 2,000 last instar larvae are harvested in one month kept in conditioned room at 30 ± 2 °C (Prasad et al. 2008). With the optimum yield of 23,926 *S. carpocapsae* IJs/larva in the present study, a total of 104,489 *C. cephalonica* larvae would be required (≈ culture obtained from 52 boxes) to produce standard nematode dosage ($2.5 \times 10^9$ IJs/ha). Therefore, the total cost of production of *Corcyra* alone comes to about 1,950 Indian rupees excluding labour charges.

Similarly, *G. mellonella* are reared in semi-synthetic diet of high calorific value placed in wooden box from which approx. 500 last instar larvae are harvested (Prasad et al. 2008). In the present investigation, an average yield of $1.6 \times 10^5$ *H. indica* juveniles was harvested from a single *G. mellonella* larva. Thus 15,244 *G. mellonella* larvae (cost of production Rs. 2,363; reared in 30 boxes) will be required to produce $2.5 \times 10^9$ IJs for treatment in one hectare.

Above calculations are only for one hectare requirement. To fulfill the greater demand, cottage industries could be set up, wherein more infrastructure and many workers will be needed, depending on financial budget and the expansion of the production unit, to run the nematode production technology based on *Corcyra* or *Galleria* as insect host. Further, their widespread installation will open avenues for generation of rural employment at grass root level.

# 9 Foliar application of *Steinenema masoodi*, *S. carpocapsae* and *Heterorhabditis indica* for *Helicoverpa armigera* management in chickpea


## ABSTRACT

Laboratory and field studies on bioefficacy of locally isolated entomopathogenic nematodes, *Steinernema masoodi*, *S. carpocapsae* and *Heterorhabditis indica* (Rhabditida: Heterorhabditidae) were evaluated against legume pod borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae) infesting chickpea. In laboratory bioassays, when *S. masoodi* (@ $3 \times 10^9$ infective juveniles (IJs)/ha + jaggary + glycerine + ujala)-sprayed chickpea leaves along with pods were offered soon after spray to *H. armigera* larvae, cumulative mortality of 85% was obtained at 72 h (in experiment 1) whereas in experiment 2, larval mortality declined from 95% to 75, 55 and 35% at 72 h when treated foliage were offered at 0, 1, 2 and 3 h post-spray. No larval

mortality was induced when treated foliage fed at 16 and 24 h post-spray in both the experiments. In field experiments conducted at Kanpur and Meerut, reduced pod damage up to 12 and 11% were obtained by the foliar application of *S. carpocapsae* (@ 3 × $10^9$ IJs/ha + glycerine + ujala) and *H. indica* (@ 5 × $10^9$ infective juveniles (IJs)/ha + glycerine + teepol), respectively. Correspondingly, maximum grain yield of 26.85 and 21.50 q/ha were harvested by utilizing above dosages resulting in 42 and 47% increase in yield over the untreated control.

## 9.1 Introduction

Among microbial control agents, entomopathogenic nematodes of the genus *Steinernema* and *Heterorhabditis* (Rhabditida: Steinernematidae, Heterorhabditidae) have been applied as foliar spray to control insect pests feeding on aboveground parts (Glazer & Navon 1989; Arthurs et al. 2004; Shapiro-Ilan et al. 2006). Poor to moderate levels of suppression were achieved when nematodes were applied to foliage to control *Helicovepra* (Bong & Sikorowski 1983; Richter & Fuxa 1990; Vyas et al. 2003). Use of nematodes to control insect pests on the foliage presents a considerable challenge because of rapid desiccation, lethal UV light and perhaps difficulty in establishing attraction gradients (Glazer 1992). Yet, under the right conditions (i.e., high humidity and during the early morning or evening), *S. carpocapsae* was reported to be effective against beet armyworms (Glazer 1992).

Recently, indigenous entomopathogenic nematodes, *S. masoodi* and *S. carpocapsae* were isolated from soils of Kanpur whereas *H. indica* was baited out from Meerut, India. Laboratory bioassays showed high efficacy of these

nematodes against *H. armigera* (unpublished data). Therefore, the present investigation was tested to evaluate their effectiveness in laboratory and field at Kanpur and Meerut. By exploitation of these nematodes supplemented with antidesiccant and UV retardant, we obtained reduced pod damage leading to increased chickpea production in comparison to control treatment.

## 9.2 Materials and methods

### *9.2.1 Insects and nematodes culture*

Greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), required for in vivo production of entomopathogenic nematodes, was reared on semi-synthetic diet as per method of Ali et al. (2005b). The test insect, *H. armigera* larvae were collected from pigeonpea/ chickpea fields and reared on semi-synthetic diet (Ali et al. 2005b) and was cultured in laboratory and 12-days old healthy larvae were chosen and used in the laboratory experiments.

*Steinernema carpocapsae* Kanpur isolate was procured from Dr. S.S. Hussaini at Section of Nematology, Project Directorate of Biological Control, Bangalore whereas *S. masoodi* and *H. indica* was isolated from soils at Kanpur and Meerut, respectively. These nematodes were mass produced on *G. mellonella* as per procedure of Kaya & Stock (1997) and applied in chickpea field within a week of nematode harvest.

### *9.2.2 Laboratory experiments*

Foliar application of *S. masoodi* was applied on chickpea variety SAKI 95–16 at fruiting stage at Indian Institute of Pulses Research (IIPR), Kanpur during *rabi* 2005-06. Liquid nematode suspension containing 7500 IJs/ml + jaggary 0.5% + glycerine 1% + Ujala 0.01% as UV retardant was prepared in water

spray of 360 ml. Sodium bicarbonate (0.5%) was also added to nullify the harmful effect of malic acid on chickpea foliage. Liquid suspension was sprayed @ $3 \times 10^9$ IJs/ha using hand compression sprayer in demarcated chickpea field (18 $m^2$) whereas in another plot (9 $m^2$) only distilled water was applied and used as control. *S. masoodi*-sprayed chickpea leaves along with pods were cut, brought in laboratory (30 ± 1°C) and offered to third instar of *H. armigera* larva kept in a plastic container (dia. 45 × 70 mm) at 0 (immediately), 16 and 24 h post-spray in experiment 1 and at 0, 1, 2, 3, 16 and 24 h post-spray in experiment 2. There were 6 treatments having 6 replications in each. Larval mortality was checked at 24, 48 and 72 h of *H. armigera* introduction. The dead larvae were removed and kept separately on moist traps to observe the emergence of nematode. Only cadavers showing emergence of juveniles were recorded as nematode induced mortality.

*9.2.3 Field experiments*

To test the bioefficacy of *S. carpocapsae* against *H. armigera* in chickpea field, foliar application was made on chickpea var. SAKI 95–16 at IIPR, Kanpur during *rabi* 2006-07. Liquid suspension contained *S. carpocapsae* IJs, 1% glycerine, 0.01% Ujala and 0.5% sodium bicarbonate. Three nematode applications were made at 10-days interval (dt. 8, 20 and 30 March 2007) during fruiting and podding stage using hand compression sprayer in chickpea field (plot size: 2 × 2 $m^2$ with 0.5 m buffer) during evening hours. There were three treatments (1, 2 or 3 × $10^9$ IJs/ha) having four replications in each. In the control set, only distilled water was sprayed.

Another field trial was set up at Crop Research Centre, Sardar Vallabh Bhai Patel University of Agriculture & Technology, Meerut during *rabi* 2007-

08. The chickpea var. Sadbhawana was grown by adopting recommended package of practices to raise a good crop. This time, only two foliar applications of *H. indica* were given with knapsack sprayer; first (dt. 25 March 2008) at the onset of *H. armigera* larval incidence in field at flowering and podding stage whereas second spray (dt. 11 April 2008) was given on 17$^{th}$ day of the first application during evening hours. The experiment was laid down in randomized block design (plot size: 4 × 5 m$^2$; 1 m buffer) having ten treatments with three replications each. The treatments consisted of 3, 4 and 5 × 10$^9$ IJs/ha with 0.2% glycerin (anti-desiccant), 0.2% teepol (surfactant) or their combination along with control.

*9.2.4 Statistical analysis*

In laboratory experiments, *H. armigera* larval mortality (%) was normalized using arcsine transformation and then subjected to ANOVA analysis. Whereas in field experiments, plot-wise healthy and damaged chickpea pods were counted and pod damage (%) was calculated, and chickpea yield was converted to q/ha. The data was statistically analyzed and means were separated using LSD. Differences among means were considered significant at $P < 0.05$.


Figure 9.1. The bioefficacy of *Steinernema masoodi* against *Helicoverpa armigera* larva (A) Lab. assistant spraying liquid suspension of *S. masoodi* using hand compression sprayer in experimental plot at IIPR, Kanpur during *rabi* 2005-06. *S. masoodi*-sprayed chickpea leaves and pods were cut at different intervals of post spray, brought in laboratory and offered to 3rd instar of *H. armigera* larva. (B) Dead *H. armigera* larva on chickpea foliage.


Figure 9.2. Field bioefficacy of *Steinernema carpocapsae* against *Helicoverpa armigera* in chickpea at IIPR, Kanpur during *rabi* 2006-07. (A) Liquid suspension containing *S. carpocapsae* infective juveniles, anti-desiccant and UV retardant in flask (many of such flasks not shown), and 10-litre sprayer ready for spraying in chickpea experimental plot. (B) Dead *H. armigera* larva after scouting in the *S. carpocapsae*-sprayed field. (C) Mature chickpea field (boundry in white line) ready to harvest.

Figure 9.3. Legume pod borer, *Helicoverpa armigera*, damaging the chickpea crop in control plots at Crop Research Centre, Sardar Vallabh Bhai Patel University of Agriculture & Technology (SVBPUA&T), Modipuram, Meerut.


Figure 9.4. Field bioefficacy of *Heterorhabditis indica* against *Helicoverpa armigera* in chickpea at SVBPUA&T, Meerut during *rabi* 2007-08. (A) Freshly harvested *H. indica* infective juveniles. (B) Diluted nematode dosage pouring in flask and then (C) in knapsack sprayer. (D) Field labourer spraying liquid suspension of *H. indica* using knappack sprayer in experimental plot.

## 9.3 Results and discussion

### *9.3.1 Laboratory experiments*

In experiment 1, when *S. masoodi*-sprayed chickpea leaves/pod was offered soon after spray, 85% larval mortality was recorded at 72 h. Whereas in experiment 2, larval mortality declined gradually from 95% to 75, 55 and 35% when treated foliage were offered at 1, 2 and 3 h post-spray (Figure 9.5). No larval mortality was induced when leaves/pods fed at 16 and 24 h post-spray in both the experiments. Result indicated that *S. masoodi* treatments were effective for initial 3 hours only. Thereafter, nematodes got desiccated over the surface of foliage hampering nematode's mobility, viability and effectiveness in killing *H. armigera* larvae. This corroborates the earlier report of short span of nematode viability over chickpea foliage (Ahmad et al. 2006).

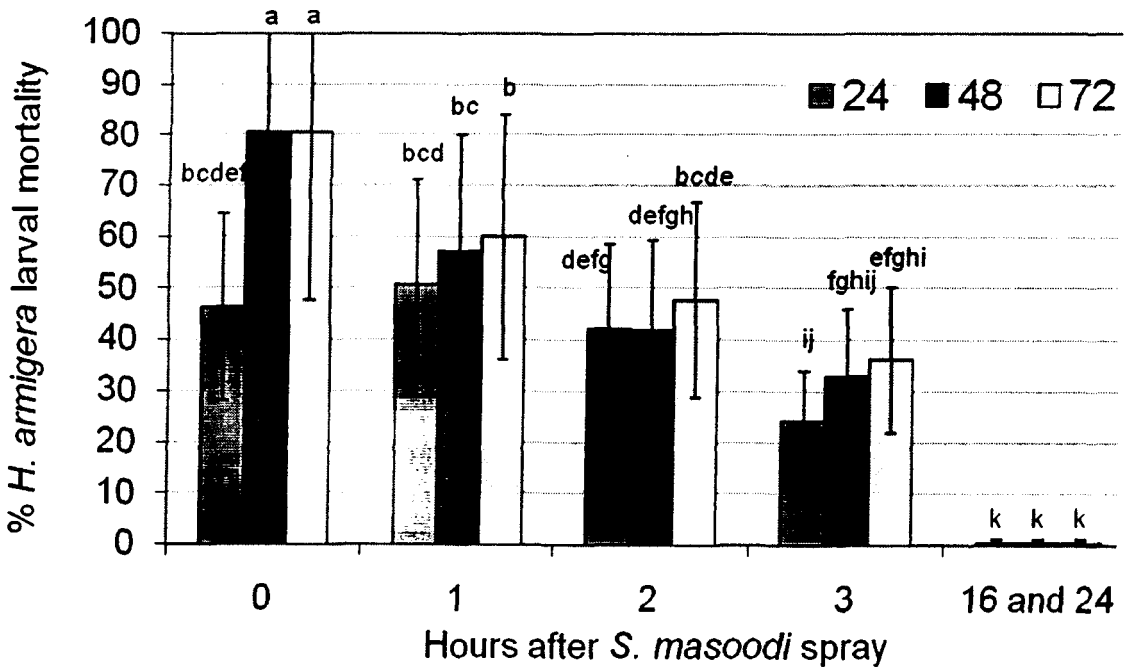



Figure 9.5. Efficacy of *Steinernema masoodi* in killing *Helicoverpa armigera* fed on *S. masoodi*-sprayed chickpea foliage. Legends (24, 48 and 72) show the hours after *S. masoodi*-treated chickpea twigs fed to *H. armigera* larvae. Bars ($n$ = 6) indicated with the same letters are not significantly different according to LSD test at $P < 0.05$. Error bars indicate the standard errors of the means.

*9.3.2 Field experiments*

In field experiment at Kanpur, highly significant results were obtained with respect to % pod damage ($F$ = 15.29; df = 3,9; $P$ = 0.001) and grain yield ($F$ = 13.83; df = 3,9; $P$ = 0.001) (Table 9.1). The lowest pod damage (12%) was recorded by the foliar application of *S. carpocapsae* @ 3 × $10^9$ IJs/ha + antidesiccant + UV retardant followed by 14, 23 and 25% pod damage by treatment of 2 and 1 × $10^9$ IJs/ha, and control, respectively. With the application of 3 × $10^9$ IJs/ha, chickpea yield of 26.85 q/ha was obtained resulting in 41.76% increased yield over the untreated control (Table 9.1).

Table 9.1. Field application of *Steinernema carpocapsae* for the management of *Helicoverpa armigera* in chickpea at Kanpur during *rabi* 2006-07.

| Treatments | Pod damage* (%) ± SE | Yield* (q/ha) ± SE | Increase over control |
|---|---|---|---|
| 1 × $10^9$ IJs/ha + glycerine 1% + Ujala 0.01% | 23.30$^b$ ± 1.07 | 20.14$^b$ ± 0.78 | 1.20 |
| 2 × $10^9$ IJs/ha + glycerine 1% + Ujala 0.01% | 14.88$^a$ ± 1.82 | 25.91$^a$ ± 0.61 | 6.96 |
| 3 × $10^9$ IJs/ha + glycerine 1% + Ujala 0.01% | 12.05$^a$ ± 0.91 | 26.85$^a$ ± 1.21 | 7.91 |
| Control | 24.83$^b$ ± 2.22 | 18.94$^b$ ± 1.31 | - |
| CD at 5% | 5.12 | 3.44 | - |

* Within a column, means ($n$ = 4) followed by the same lowercase letter are not significantly different at $P < 0.05$.

By the foliar application of *H. indica* at Meerut, highly significant results were obtained with respect to % pod damage ($F = 2.19$; df = 2,18; $P < 0.0001$) (Table 9.2). Out of the nematode dosages applied, least pod damage (11%) was achieved by the application of $5 \times 10^9$ IJs/ha + glycerin + teepol whereas highest pod damage (28%) was accounted in treatment $4 \times 10^9$ IJs + teepol followed by control (29%). With regard to chickpea yield, statistically significant results ($F = 0.65$; df = 2,18; $P = 0.003$) were obtained. Highest yield of 21.5 q/ha was gained by treatment of $5 \times 10^9$ IJs/ha + glycerin + teepol whereas lowest (16.8 q/ha) was recorded in the application of $4 \times 10^9$ IJs/ha + teepol followed by control (14.6 q/ha). Overall, nematode treatment (3 to 5 $\times 10^9$ IJs/ha) combined with glycerin + teepol were better than use of *H. indica* + glycerine or teepol alone (Table 9.2). In the present study, greater level of control was not achieved as reported by Vyas et al. (2002) in pigeonpea field trial. They reported reduced larval population of *H. armigera* by 16.7 and 28.5% over the initial population when *Heterorhabditis* sp. was sprayed alone (@ $1 \times 10^9$ IJs/ha) and with adjuvants (5% starch + gum arabic), respectively.

Patel & Vyas (1995) obtained 24.6% mortality of *H. armigera* after 6 days of *S. glaseri* spray @ 200 IJs/ml on chickpea in pots. However, Vyas et al. (2003) recorded 59 and 71% larval mortality on $4^{th}$ and $6^{th}$ day of spray of *Heterorhabditis* sp. @ 2000 IJs/pot, respectively and reported increased yield of chickpea. The effectiveness of *S. carpocapsae* against *H. armigera* on cotton @ 5000 IJs/ml was reported to be a low 22% in distilled water (Glazer et al. 1992). However, they observed a high mortality of 85-95% when

antidesiccants like Biosys 627, natural wax and Folicote were added in spray suspension.

Table 9.2. Field application of *Heterorhabditis indica* for the management of *Helicoverpa armigera* in chickpea at Meerut during *rabi* 2007-08.

| Treatments | Pod damage* (%) ± SE | Yield* (q/ha) ± SE | Increase over control |
|---|---|---|---|
| $3 \times 10^9$ IJs/ha + glycerine 0.2% | $18.24^{cd}$ ± 1.03 | $18.42^{bc}$ ± 0.89 | 3.81 |
| $4 \times 10^9$ IJs/ha + glycerine 0.2% | $20.75^{de}$ ± 0.89 | $18.76^{abc}$ ± 1.05 | 4.15 |
| $5 \times 10^9$ IJs/ha + glycerine 0.2% | $18.78^{cd}$ ± 1.47 | $19.20^{abc}$ ± 0.81 | 4.59 |
| $3 \times 10^9$ IJs/ha + Teepol 0.2% | $22.76^{ef}$ ± 1.68 | $18.10^{bc}$ ± 0.76 | 4.94 |
| $4 \times 10^9$ IJs/ha + Teepol 0.2% | $28.31^{gh}$ ± 1.30 | $16.85^{cd}$ ± 0.88 | 2.24 |
| $5 \times 10^9$ IJs/ha + Teepol 0.2% | $25.24^{fg}$ ± 0.75 | $18.95^{abc}$ ± 0.70 | 4.34 |
| $3 \times 10^9$ IJs/ha + glycerine 0.2% + Teepol 0.2% | $13.26^{abc}$ ± 1.06 | $20.08^{ab}$ ± 0.67 | 5.47 |
| $4 \times 10^9$ IJs/ha + glycerine 0.2% + Teepol 0.2% | $15.95^{bcd}$ ± 0.63 | $20.20^{ab}$ ± 1.53 | 5.59 |
| $5 \times 10^9$ IJs/ha + glycerine 0.2% + Teepol 0.2% | $11.04^{ab}$ ± 1.27 | $21.50^{a}$ ± 0.86 | 6.89 |
| Control | $29.21^{h}$ ± 1.82 | $14.61^{d}$ ± 0.56 | |
| CD at 5% | 3.5 | 2.75 | - |

* Within a column, means followed by the same lowercase letter are not significantly different at $P < 0.05$.

Further studies on enhancement of viability of entomopathogenic nematodes with adjuvant, antidesiccant or humectant are warranted, which could increase their survival and efficacy under field conditions. The present study indicated that *S. carpocapsae* and *H. indica* could be used in the management of *H. armigera* but suitable antidesiccant(s) and/or adjuvant(s) have to be incorporated in the spray solution to increase the survival and efficacy of the nematode.

## Summary

The legume/gram pod borer (also known as American or cotton bollworm), *Helicoverpa* (= *Heliothis*) *armigera* (Lepidoptera: Noctuidae), is a polyphagous insect pest distributed over tropics and subtropics of the world. The economic damage has been recorded in agriculturally important crops such as cotton, chickpea, pigeonpea, maize and a range of oilseeds, vegetables, and fruit crops. In recent times, crop production has been severely threatened by the increasing difficulties in controlling *H. armigera* as it has developed high levels of resistance to commonly used insecticides. Use of integrated pest management (IPM) strategies can reduce existing over-dependence on insecticides and their negative effects on the environment. Manipulation of cultural practices, host-plant resistance and biological control can play a crucial role in reducing the ravages caused by *H. armigera*. Biological control involving natural enemies and biopesticides has shown some promise in reducing *Helicoverpa* damage. Efforts to develop *Helicoverpa*-resistant cultivars have been ongoing for the past two decades, but the progress has been very limited mainly due to low levels of resistance in the cultivated germplasm. Genetic tansformation and marker-assisted selection have enabled the transfer of genes from the same and unrelated species, which have shown a great promise in pest management. At present, environmentally safe technologies such as use of natural enemies, pheromones, natural plant products and biopesticides are not as yet in a deliverable form that can be adopted by farmers on a large scale. Mass production, formulation, storage & delivery, and to a certain extent, bioefficacy and economics have hampered large-scale adoption of alternate methods of control.

Among microbial control agents, entomopathogenic nematodes of the genus *Steinernema* and *Heterorhabditis* (Rhabditida: Steinernematidae, Heterorhabditidae) have been used against insects encompassing several insect Orders. These nematodes have been applied as foliar spray to control insect pests feeding on above-ground parts. Several studies have been reported the susceptibility of *H. armigera* to *Steinernema* and *Heterorhabditis*. These nematodes can also lower the dependence on conventional insecticides to manage pest populations in refuges. Keeping the above information and prospects in view, overall objective of the present investigation was to evaluate the effectiveness of locally isolated nematodes in controlling *H. armigera* in laboratory and field.

To begin with, studies on pathogenicity, development and reproductive potential of locally isolated *Steinernema masoodi* in *H. armigera* were carried out under laboratory conditions (Chapter 3). In filter paper bioassay, cumulative mortality of different larval instars of *H. armigera* ranged 28 to 86% after 72 h exposure of *S. masoodi* infective juveniles (IJs). Third and fourth instar larvae were more susceptible than second and fifth instar. Average progeny produced was 124,280 IJs/larva and overall 216,951 IJs/g of body weight of larva was obtained. Further experiments were conducted to access the susceptibility of *H. armigera*, *Galleria mellonella* and *Corcyra cephalonica* (Lepidoptera: Pyralidae) to *S. masoodi*, *S. seemae*, *S. carpocapsae*, *S. glaseri* and *S. thermophilum* (Chapter 4). All the nematode species studied killed the test insects within 48 h except *S. thermophilum*, which took 56 h to kill *H. armigera*. *Galleria mellonella* was the most suitable host for mass production of *S. seemae* by producing higher yield than *S. carpocapsae*,

whereas, *H. armigera* was next best suitable alternate host followed by *C. cephalonica*.

Mortality and/or reduced infectivity of entomopathogenic nematodes under field conditions are one of the most important factors restricting their application in subtropical ecosystems where temperature can be high. Generally, nematodes become sluggish at lower temperatures (< 10 – 15°C) and inactivated at higher temperatures (> 30 – 40°C). Therefore, laboratory investigations on the effect of temperature exposure (ranging from 15 to 45°C for 6 h treatment) on survival of *S. masoodi*, *S. seemae* and *S. carpocapsae* was undertaken to determine the upper limit of heat tolerance and infectivity of survived nematodes to prepupa of *H. armigera* (Chapter 5). Percent survival of nematodes decreased with increase in temperature, neverthless, 47% populations were able to tolerate sub-lethal temperature (45°C). Out of the populations that survived, 43% infectivity was observed against *H. armigera* prepupa. Though both the species, *S. seemae* and *S. masoodi*, have been isolated from regions of high temperature zone, but the former seems to tolerate better than the later, so much so that at 45°C, its infectivity was 47% against 36% in *S. masoodi* and *S. carpocapsae*.

The survival of *S. masoodi* on pigeonpea and *S. carpocapsae* on chickpea foliage were investigated after foliar spray of nematodes in liquid suspension containing UV retardant (fabric whitener) and anti-desiccant (Chapter 6). *S. masoodi* survival was 17% on pigeonpea foliage after 1 h of spray during evening hours as compared to 3% survival in morning spray. In chickpea, *S. carpocapsae* survival was 40 and 70% after 1 h post-spray during evening and morning hours, respectively. Within 3 h of spray, there was drastic

reduction in survival of *S. carpocapsae* population. The results indicated that anti-desiccant and UV retardant was not very effective in protecting the nematode survival beyond 3 h in morning but effective to some extent in evening hours. Further studies are warranted to find new molecule(s) acting as adjuvant, humectant, anti-desiccant and/or UV retardant, which could be incorporated in spray solution to prolong nematode survival on foliage and fruiting bodies.

The foliar application of nematode can be one strategy to control *H. armigera* feeding on above-ground foliage and fruiting parts. However, other possibility to curb further *H. armigera* population is the upper soil profile, the very own habitat of entomopathogenic nematodes, where the final instar larva after completion of larval stage fall to ground and enters into crevices or loose soil for pupation in earthen pupal case. There is likelihood that such larva may encounter nematode present in soil and get killed prior to metamorphosis into next developmental stage, i.e. pupa or adult. Therefore, the evaluation of *S. masoodi* was carried out against soil-dwelling stage of *H. armigera* in laboratory and microplot (Chapter 7). In laboratory, suppression of 71 ± 10% adult emergence was obtained at nematode concentrations of 500 to 5,000 IJs/60g soil/larva. At lower nematode concentration (at 450 IJs/larva), adult suppression of 64 ± 7% was recorded, whereas, lowest (30 ± 3%) being at 50 IJs/larva. In chickpea microplots (40 × 40 $cm^2$), adult suppression of 70 ± 2% and 56 ± 3% were obtained by the application of *S. masoodi* @ 6.0 and 4.0 × $10^9$ IJs/ha, respectively.

The mass production of entomopathogenic nematodes can be done in vivo (in insect hosts) or in vitro (solid or liquid) culture methods. Although *in vitro*

production has the advantage of economy and large quantity production, neverthless in vivo culture is still essential to numerous scientific and industrial interests. In vivo method is suitable for laboratory-scale production and for generating materials for field trial. Experiments were conducted to optimize the production of local isolates of *S. carpocapsae* and *H. indica* in *C. cephalonica* and *G. mellonella* larvae, respectively at 28°C (Chapter 8). The effect of cadaver density on final yield and economics of nematode production was also worked out. The average production of 31,907 IJs/larva was obtained from 1.5g *C. cephalonica* cadavers placed on modifield White trap followed by 23,926 (2.0g), 14,738 (1.0g), 7,533 (3.0g) and 4,845 IJs/larva (6.0g). The results obtained showed that overcrowding of cadavers had a negative impact on final yield. A total of 6,131 $\times$ $10^5$ IJs of *H. indica* were harvested from 3,834 *G. mellonella* larvae (average: 1.6 $\times$ $10^5$ IJs/larva). Economics of 2.5 $\times$ $10^9$ IJs of *S. carpocapsae* production was calculated as Rs. 1,950 (≈ US$ 39), whereas *H. indica* from *G. mellonella* was Rs. 2,363 (≈ US$ 47) excluding cost on labour. If nematode alone is used in crop protection then it is bearable, but application cost becomes costly by inclusion of expenditure incurred on other control measures when used together. Therefore, further studies are needed to work out ways and means to reduce the cost of nematode production.

Finally, laboratory and field studies on bioefficacy of *S. masoodi*, *S. carpocapsae* and *H. indica* were evaluated against *H. armigera* infesting chickpea (Chapter 9). In field experiments conducted at Kanpur and Meerut, chickpea pod damage was reduced up to 12 and 11% by foliar application of *S. carpocapsae* and *H. indica*, respectively by addition of anti-desiccant and

UV retardant in spray solution. Correspondingly, maximum grain yield of 27 and 21 q/ha were harvested by utilizing above dosages resulting in 42 and 47% increase in yield over the untreated control. This level of control is not acceptable by end users, still a higher level of control is desirable which was not achieved by use of nematode alone.

In conclusion, variable efficacies of indigenous entomopathogenic nematodes, viz., *S. masoodi*, *S. carpocapsae* and *H. indica* were obtained in laboratory tests against *H. armigera*. However in field, the survival of *S. masoodi* and *S. carpocapsae* on pigeonpea and chickpea foliage, respectively remained up to few hours only. As a consequence, *H. armigera* escape nematode's pathogenic attack and continue damaging fruiting bodies. Results of foliar applications in field indicated that nematode alone is not effective in controlling *H. armigera*. Integration of other biological control agents like parasitoids, *H. armigera* nucleopolyhedrovirus (*Ha*NPV), *Bacillus thuringiensis* (*Bt*), neem seed kernel extract (NSKE) and/or their combinations should be attempted for better control of damaging stage of *H. armigera* population in standing crop. The other novel strategy emerging out of the present study is the use of additional control measure against pupating larvae in soil by innundative release of nematode at appropriate time for a wider area and diverse cropping system where *H. armigera* is the pest for most of the crops grown. Charging nematode in irrigation water could substantiate an approach to suppress the forthcoming generations of *H. armigera* in chickpea; thus preventing adult emergence, subsequent migration and causing damage to succeeding chickpea, pigeonpea, cotton or other host crops.

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